Vulnerability analysis of the rare woodland fern *Polystichum braunii* (Dryopteridaceae) in Germany

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List of abbreviations

AA	Allgäu Alps
ABG	Aboveground biomass
Al	Aluminium
BA	Bavarian Forest
$BaCl_2$	Barium chloride
BF	Southern Black Forest
BGB	Belowground biomass
C	Carbon
Ca	Calcium
Ca/Al	Ratio of the mass of calcium to the mass of aluminium
CEC	Cation exchange capacity
C/N	Ratio of the mass of carbon to the mass of nitrogen
C_{org}/N_t	Ratio to the mass of organic carbon to the mass of total nitrogen
F	Percentage fertile frond section
FED	Percentage ferns with leaf deformities
FL	Frond length
$^1H/^2H$	Ratio of the mass of hydrogen to the mass of heavy hydrogen
^2H	Heavy hydrogen

$2H_2O$	Heavy water
HE	Northern Hesse
LC	Leaf conductance
N	Nitrogen
NaCl	Sodium cloride
NaOH	Sodium hydroxide
NOT	Number of trichomes
NSB	Percentage not sprouted buds
N_t	Total nitrogen
P	Phosphorus
PPFD	Photosynthetically active radiation
P_{resin}	Plant available phosphors
RH	Relative air humidity
RSR	Root per shoot biomass ratio
SA	Saxon Uplands
SLA	Specific leaf area
SM	Soil moisture
TB	Total biomass
VPD	Vapor pressure deficit

CHAPTER 1

General Introduction

Background

The species extinction rate in the Anthropocene is estimated to be 100 to 1000 times higher than it is probably considered to occur naturally and it has proved to affect the earth ecosystems as strongly as climate warming, ozone depletion, nutrient pollution or changes in land use (Rockström et al. 2009, Hooper et al. 2012). The most dramatic species losses have been recorded for the tropical forest regions where intensive logging or slash-and-burn agriculture has been responsible for the extirpation of a huge quantity of endemic plant and animal life (cf. International Union for Conservation of Nature and Natural Resources, 2016). Unfortunately, this process has not been slowed down since the governments of the world committed themselves to the Convention on Biological Diversity of Rio in 1992 (Sala et al. 2000, Butchart et al. 2010). One of the main reasons for this is the fact that the main drivers responsible for extinctions are still in place (Bucella 2011). Another cause is the lack of knowledge about the ecology of many rare and/or endangered species (cf. Schnittler et al. 1998, IUCN 2016). A better understanding of the later, for example, should help to prevent habitat distraction and/or to develop adequate protective measures.

In Central Europe, one of the world's highly industrialized areas, the preservation of plant biodiversity is one of the main issues of conservation (Schnittler and Günther 1999). Intensive land use and widespread eutrophication since the 1970´s have led to a particular decrease in the number of phanerogams (e.g. Tilman et al. 2001, Krauss et al. 2010, Meyer et al. 2015). It is supposed that about 40% of the vascular plants in Europe are now endangered (Bilz et al. 2011). Repeated surveys, monitoring data and consecutive assessments of red-listed species suggest that loss of species is generally less pronounced in forests than in the non-forest vegetation of Central Europe (Korneck et al. 1998). The diversity in the herbaceous layer of forest floors has indeed remained stable or even increased in much forest vegetation which has been monitored over decades. Further data analyses, however, have revealed distinct shifts within forest communities from rather characteristic woodland specialists which depend on stand continuity towards more generalist species and invasive neophytes which also occur at forest edges or in the open and benefit from clearings in highly managed forests (Lameire et al. 2000, Jantsch et al. 2013). Dramatic losses in the woodland species pool e.g. have been recorded for the lichen and bryophyte flora of Central European forests during the last 50 to 150 years (Hauck et al. 2013, Dittrich et al. 2014). The latter fact is thought to be caused by the disappearance of old-growth forests with the presence of old trees and dead wood, the shift to high-forest management with more closed canopies, nitrogen and acid deposition, and (in the case of bryophytes) an apparent decrease in air humidity, due to widespread drainage since the Middle Ages (Koperski 1998, Ellenberg and Leuschner 2010).

Ferns are conspicuous components of forest vegetation particularly in moist climates, thriving in high air humidity and on moist soil in the herbaceous layer and, in warmer climates, as epiphytes on trees. About 1,300 species occur worldwide (the Plant List 2016) while about 100 species are indigenous in Central Europe (Hegi 1984). In Germany, about 50% of the ferns and fern-allies are now being threatened (Federal Agency for Nature Conservation 2016). Many of these have suffered massive population losses within the past few decades or even entirely disappeared from some regions (cf. Bennert 1999, Jessen 2009). Some of the factors that decimated the lichen and bryophyte communities and led to the shifts from woodland specialist towards more generalists certainly also affected the pteridophyte flora in Central European woodlands. The causes for the fading of many rare and endangered ferns are not yet well understood which is also the case in other regions of the world (e.g. Kelly 1994, Rünk et al. 2004, Watkins et al. 2007, Bucharová et al. 2010). It may be speculated that this is probably linked to the - in part surprisingly - poor knowledge about the ecology of this group. A global comprehensive study of elevation gradient data from Kessler et al. (2011) proved that beside temperature, water availability is a driving factor for the diversity and distribution of ferns. It is, however, not well known whether soil moisture availability or the atmospheric water status, are decisive factors, or if both are equally important.

The fronds of ferns grow through the division of an apical meristematic cell or a cell group which is active for a long time, resulting in continued leaf growth. A key role in cell division and cell expansion processes is played by the water status of these frond meristems. Since the fronds are situated on the way the water takes flowing from the soil through the plant into the atmosphere, it seems logical that not only soil moisture (and precipitation) but also atmospheric moisture status influence frond growth, independently regardless of soil moisture. High air humidity could for example increase leaf water relations due to a reduction of the transpirative water loss. The positive effect of low VPD levels on plant growth has been demonstrated by factorial climatic chamber experiments for a number of herbaceous plants and woody angiosperms (Leuschner 2002, Lendzion and Leuschner 2009) while similar experiments concerning the single influence of air and soil moisture or temperature on the morphogenesis and productivity of ferns are still lacking. Many vascular plants including trees, scrubs and herbal species of rather humid environments were further found to improve their water status through foliar water uptake (e.g. Stone 1950, Katz et al. 1989, Grammatikopoulos and Manetas 1994, Limm et al. 2009, Goldsmith 2013). However, only very few pteridophytes have been investigated regarding their ability to absorb liquid water via the epidermal leaf surface (c.f. Pessin 1924, Stuart 1968, Matthes-Sears, Kelly and Larson 1993, Limm et al. 2009). The morphological and chemical properties of the cuticle influence the wettability of the leaf surface (Koch and Barthlott 2009) and they should also determine how much water can be absorbed by a leaf. The cuticle's permeability for water has proved to vary depending of the ecology of different plant species (e.g. evergreen, deciduous or scleromorphic) and their occurrence in different climates (Schreiber and Riederer 1996, Limm and Dawson 2010). In addition, trichomes could also influence how much water penetrates the leaf surface. Certain trichomes appear to retain the water on the leaf surface, thereby probably reducing the evapotranspiration rate and/or the wettability (Grammatikopoulos and Manetas 1994, Munné-Bosch 2010, Fernández et al. 2014). Nevertheless, the functions of trichomes in ferns, which show considerable morphological variability among different species, are still mostly unclear (Halloy and Mark 1996, Watkins 2006, Kluge and Kessler 2007). The fact that poikilohydric ferns often exhibit high trichome densities (Kessler and Siorak 2007) suggests that these laminar structures play a certain role in the water status of the fronds (e.g. Pessin 1924, Stuart 1968, Müller et al. 1981, Tsutsumi and Kato 2008). A recent worldwide assessment of temperature and precipitation effects on plants further led to the conclusion that a distinction between soil and atmospheric water status effects will urgently be needed in future scientific studies (Moles et al. 2014) particularly with respect to the predicted alterations of the global climate. In Central Europe summer temperature increase by 4 K have been predicted until the end of the 21st century (Beniston 2004, IPCC 2013) with more frequent summer heat waves and higher frequency of dry periods (Schär et al. 2004).

In this study, we aimed at exploring the ecology of *Polystichum braunii*, one of the rarest woodland ferns in Central Europe with only about 15 remaining sites, four of them being located in Germany. Here, at least 10 populations have become extinct during the last 50 years (cf. Bennert 1999, Baier et al. 2005, Jessen 2009, Wildlife Conservation Program Baden-Württemberg) while 50% of the remaining populations consist of <10 individuals today. The German Government has recognized this situation and put the species on the national red list (category 2+ = highly endangered). A decline of the species is also known from other regions in the world, that is considered as highly endangered in several countries (e.g. in Germany: Federal Agency for Nature Conservation; Switzerland: The National Data and Information Centre of the Swiss Flora; France: Botanical Conservation Agency of Franche-Comté). Two of the German *P. braunii* sites still harbor larger populations (in the Southern Black Forest and the Allgäu Alps) with up to 400 individuals which results in a particular responsibility of the German authorities for the global protection of this species because populations are generally not larger than 100 individuals (Brzeskiewicz and Field 2003).

The genus *Polystichum* (Dryopteridaceae) contains about 260 species worldwide most of which occur in Eastern Asia (about 100) while only four are indigenous in Central Europe (*Polystichum aculeatum*, *Polystichum braunii*, *Polystichum lonchitis* and *Polystichum setiferum*). A general morphological characteristic of the genus is the shape of the leaflet and/or second-order pinnae which show a typical and more expanded base towards one side, called auricle (marked red in Fig. 1.1a). Hybridization is common in this genus. That is why species determination can be difficult in some cases (cf. Czichowski and Hilmer 2010). A significant characteristic of *P. braunii* is represented by the dense filamentous trichomes on both sides of the second-order pinnae (Fig. 1.1a). *P. braunii* is an allotetraploid fern (2n = 164). The rhizome is short and the fronds are arranged in a circle which can be up to 100-120 cm long (Fig. 1.1b and c). The rather soft and tenuous, sometimes shiny leaves stay green during the winter. *P. braunii* is widespread in the temperate and boreal zones of Eurasia and North America, however regionally rare and highly patchily distributed while most of the populations are separated from each other by hundreds to thousands of km (Hulten 1961, Wagner 1993). In Germany the species occurs within shady, steep, rocky ravine forests in the middle mountain range and the Alps where the plants grow on shallow soil and on mossy rocks (Fig. 1.1b-f). Populations are often found close to mountain streams and occasionally next to waterfalls (Fig. 1.1c). The bedrock is mainly siliceous, the soil layers are only thin (Fig. 1.1d) but nutrient rich, caused by the continuous supply of water running down and the fast turn-over rates in the lower organic layer horizons (Of and Oh layers) (Ellenberg and Leuschner 2010). These azonal habitats are ravine ash-maple-linden forests, notably the Fraxino-Aceretum pseudoplatani, the Ulmo-Aceretum pseudoplatani and the Aceri-Fagetum associations. *Impatiens noli-tangere, Circaea lutetiana, Chrysosplenium alternifolium* and *C. oppositifolium* as well as *Oxalis acetosella* indicate humid growing conditions.

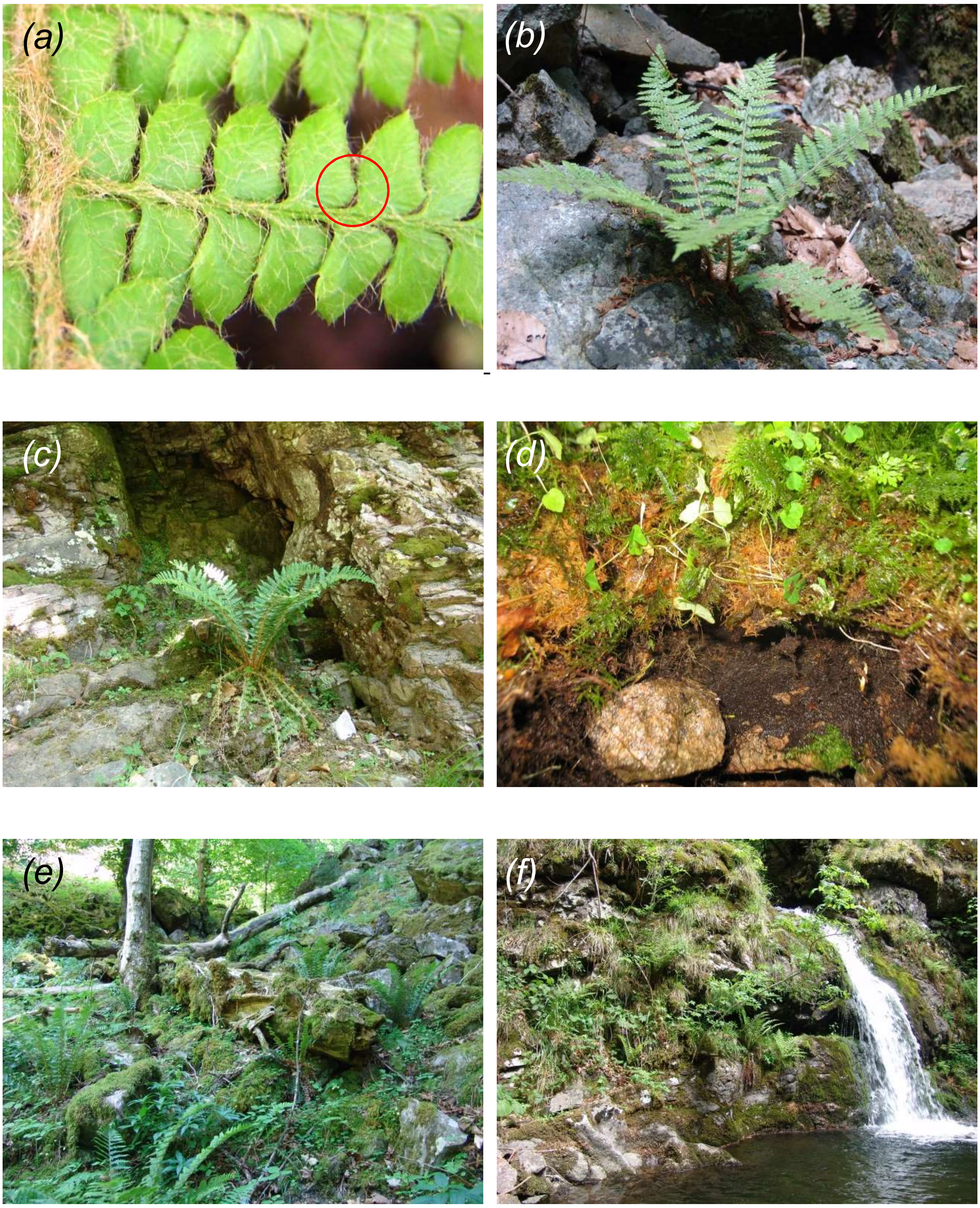

Figure 1.1. Illustration of the habit and the habitats of *P. braunii*. Auricles characteristic of the genus (marked red), and leaf trichomes (a), plant habit (b, c), characteristic soil profile (d) and habitat examples (e, f).

There has been some discussion on the possible causes for the decline, including logging operations, trampling in context of recreation activities, herbivory, poaching and competition with invasive species (e.g. Sebald et al. 1993, Baier et al. 2005, Henneqion 2006). As regarding the German populations, however, it is not likely that these factors are decisive factors of the proceeding transregional losses, because all the populations are located within conservation areas. The very limited extension of humid ravine forests, in which most Central European populations of *P. braunii* are thriving, may be a factor which limits the population size (Bennert et al. 1999). It is however not known, which site factors are responsible for the apparent dependence of the fern on this specific habitat. There has been some speculation about the fact that alterations in the local hydrological regimes, changes in winter snow cover and climate warming could be factors affecting the species negatively (Eberle 1966, Rasbach et al. 1976, Mütter et al. 1998, Brzeskiewicz and Field 2003, Jessen 2009). Sometimes the reasons for species extinction are clear, for example in the case of habitat loss due to mining or forest engineering. In other cases they can be more difficult to understand: Gundale (2002), for example, proved a significant correlation between the abundance of an exotic earthworm and the extirpation of the rare fern *Botrychium mormo*.

Study aims and methods

Different approaches in the field and the laboratory were undertaken in this study to find hints of the possible causes of the decline of *P. braunii* in Germany and to close some of the consisting knowledge gaps concerning the ecology of woodland ferns in general. A three-factorial climate chamber experiment (soil moisture x air humidity x air temperature) with 144 plants and realistic environmental conditions was performed in order to detangle the single influences of the most important climatic growth factors for the fern. Four walk-in precision plant growth chambers (Johnson Controls, Milwaukee, WI, USA) were used to establish two temperature levels (15 and 19 °C during daytime) and two air humidity levels (95% and 65% RH) in factorial combination. The soil moisture ranged from 41.8 to 42.2 vol. % in the dry treatment, 48.5–60.5 vol. % in the mesic treatment, and 66.1–70.0 vol. % in the moist treatment. We defined the temperature, soil moisture and air humidity ranges in the experiment using microclimate measurements at three *P. braunii* sites in Germany, and extrapolated these to the predicted thermal and hygric conditions in a future climate with warmer and drier summers. With the aim of a better understanding of the causes driving population decline and extinction in the *P. braunii* populations of Germany, we further conducted an observational study in the four remaining regions in which the species occur by investigating probably important microclimatic, edaphic and vegetation structural parameters. Based on population size data of Bennert (1999) and successive population size counts of our own in 2011 and 2014, we distinguished populations with either increasing or decreasing population trends in recent times and also considered a third category, i.e. now extinct populations. A tracer experiment with heavy hydrogen (^{2}H) was also conducted to verify if *P. braunii* and four other temperate woodland ferns (*Athyrium filix-femina*, *Dryopteris filix-mas*, *Polystichum aculeatum* and *Asplenium scolopendrium*) feature foliar water uptake and if the characteristically high frequency of epidermal leaf trichomes of *P. braunii* correlates with this capability.

The main objectives of this study can be summarized as follows:

(i) analysis of the most important climatic growth factors of the terrestrial woodland fern *P. braunii*

(ii) exploration of the causes driving population decline and extinction in the *P. braunii* populations of Germany

(iii) investigation of the ability of foliar water foliar uptake of *P. braunii* and four other temperate woodland ferns

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CHAPTER 2

Air humidity as key determinant of morphogenesis and productivity of the rare temperate woodland fern *Polystichum braunii*

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Abstract

(1) Most ferns are restricted to moist and shady habitats, but it is not known whether soil moisture or atmospheric water status are decisive limiting factors, or if both are equally important. (2) Using the rare temperate woodland fern *Polystichum braunii*, we conducted a three-factorial climate chamber experiment (soil moisture (SM) 3 air humidity (RH) 3 air temperature (T)) to test the hypotheses that: (i) atmospheric water status (RH) exerts a similarly large influence on the fern's biology as soil moisture, and (ii) both a reduction in RH and an increase in air temperature reduce vigor and growth. (3) Nine of 11 morphological, physiological and growth-related traits were significantly influenced by an increase in RH from 65% to 95%, leading to higher leaf conductance, increased above- and belowground productivity, higher fertility, more epidermal trichomes and fewer leaf deformities under high air humidity. In contrast, soil moisture variation (from 66% to 70% in the moist to ca. 42% in the dry treatment) influenced only one trait (specific leaf area), and temperature variation (15 °C versus 19 °C during daytime) only three traits (leaf conductance, root/shoot ratio, specific leaf area); RH was the only factor affecting productivity. (4) This study is the first experimental proof for a soil moisture-independent air humidity effect on the growth of terrestrial woodland ferns. *P. braunii* appears to be an air humidity hygrophyte that, within the range of realistic environmental conditions set in this study, suffers more from a reduction in RH than in soil moisture. A climate warming related increase in summer temperatures, however, seems not to directly threaten this endangered species.

Keywords: Climate chamber experiment; pteridophyte; soil moisture; temperature; vapor pressure deficit

Introduction

Ferns are a conspicuous component of forest vegetation in moist climates, thriving in high air humidity and on moist soil in the herbaceous layer and, in warmer climates, as epiphytes on trees. Most ecologists would agree that a large majority of woodland ferns depend on ample water supply, but it is not well known whether soil moisture availability or atmospheric water status are decisive factors, or if both are equally important. The relationship between pteridophyte diversity, abundance and productivity and possible environmental drivers has been investigated in several comparative studies along elevation gradients in tropical and temperate mountains (e.g. Mütter et al. 1998, Wegner et al. 2003, Bhattarai et al. 2004, Bickford and Laffan 2006, Kluge et al. 2006, Kluge and Kessler 2007, Odland et al. 2008, Creese et al. 2011, Salazar et al. 2013). A global analysis of elevation gradient data led to the conclusion that the occurrence of ferns is best predicted by water availability and temperature (Kessler et al. 2011), without specifying the role of the different water cycle components. As soil moisture and relative air humidity (RH) are generally closely related, both depending to a large extent on precipitation, it is in most cases difficult to disentangle the effects of soil and air moisture on fern vitality and growth in gradient studies. A recent worldwide assessment of temperature and precipitation effects on plants also concluded that a distinction between soil and atmospheric water status effects is needed (Moles et al. 2014). To our surprise, experimental data clarifying the importance of soil moisture, air humidity and other environmental parameters as factors influencing the biology of ferns are scarce. The fronds of ferns grow through the division of an apical meristematic cell or a cell group that is active for long time, resulting in continued leaf growth. A key role in the cell division and cell expansion processes is played by the water status of these frond meristems. Since the fronds are situated on the water flow path from the soil through the plant to the atmosphere, it seems logical that not only soil moisture (and precipitation) but also atmospheric moisture status influence frond growth, independently of soil moisture. This has been demonstrated in factorial climate chamber experiments with independent variation of soil and air moisture for a number of herbaceous and woody angiosperms (e.g. Leuschner 2002, Lendzion and Leuschner 2009), but a similar experiment for ferns is lacking. *Polystichum munitum*, a fern of the redwood forests of northern California, was found to absorb fog droplets during periods of low water availability in summer (Limm and Dawson 2010). However, there are a few studies examining the effect of soil moisture variation on fern growth without consideration of the role of air humidity (e.g. Liao et al. 2008 and 2013). This is unsatisfactory, as our understanding of the distribution and ecology of ferns should be based on a mechanistic understanding of the environmental controls of fern growth and survival, and thus a separation between the roles of soil and atmospheric factors. Moreover, recent climate warming will lead to warmer and drier summers in many regions, which could affect ferns via higher temperatures and lower soil moisture in summer, but additionally through reduced air humidity, if RH acts independently of soil moisture on the plants' vitality and growth. To close this knowledge gap, we conducted a three-factorial climate chamber experiment to quantify the influence of air temperature, relative air humidity and soil moisture and their interactions on the morphology, vigor and productivity of the circumboreal fern species *Polystichum braunii* (Spenn.) Fée. This woodland fern occurs very locally in cool shady hardwood forest habitats of the northern hemisphere. Due to its patchy distribution range and, in many regions, decreasing population sizes, the species is considered threatened or endangered in Germany (FloraWeb database of the German Office for Nature Protection, BfN), the United States and Canada (Brzeskiewicz & Field 2003). One possible cause of its rarity and recent decline is the deterioration in site water balance (Rasbach et al. 1976, Bennert 1999, Baier et al. 2005), but this assumption has not yet been rigorously tested (Mütter et al. 1998). A fully factorial growth experiment in climate chambers is the only means to separate soil and atmospheric drivers, and has the advantage over observational field studies that possible co-varying factors such as soil chemistry can be controlled. We chose *P. braunii* as a model organism because the species represents a typical temperate pteridophyte of the cool and humid forest interior that might be particularly sensitive to climate warming. Moreover, our results could help to better understand the causes of its rarity and to develop science-based conservation strategies through better knowledge of the species' habitat requirements. We tested the hypotheses that (i) atmospheric water status exerts a similarly large influence on the biology of *P. braunii* as soil water status, and (ii) both reduced air humidity and increased air temperature, relative to current climate conditions, reduce vigor and growth.

Material and methods

Plant material

The sporophytes used in the experiment were reared from spores in a glasshouse at the Botanical Garden of the University of Kassel (Germany). The spores had been collected in 2005 from a population in northern Hesse (Hoher Meißner, Central German Uplands). Hence, all plants used in the study were of the same age and had roughly similar sizes. Before the start of the experiment, frond length (mean of all plants: 42.87 ± 0.2 cm), frond width (8.87 ± 0.2 cm), percentage of fertile sections on fronds (8.93 ± 0.2%) and aboveground biomass (3.38 ± 0.2 g) of previous-year fronds were measured in all plants used for the experiment. Average belowground biomass (8.19 ± 0.5 g) was taken separately in 12 additional individuals not used in the experiment. The ferns were containerized individually in pots of 12 x 12 x 17 cm (l x w x h), each filled with an equal amount of a peat–sand mixture (70/30%) and installed in the climate chambers for acclimation 4 weeks before the experiment started. To avoid premature sprouting of plants, temperature was kept at 10 °C during the day and 5 °C during the night at 95% RH during the acclimation period.

Experimental design and climate variables

A total of 144 sporophytes of *P. braunii* were installed from 1 May to 30 September 2013 in the three-factorial climate chamber experiment in the Experimental Botanical Garden of Göttingen University (factors: air temperature, air humidity, soil moisture). Four walk-in precision plant growth chambers (Johnson Controls, Milwaukee, WI, USA) were used to establish two temperature levels (15 and 19 °C during daytime; labelled as ‘cool’ and ‘warm’ hereafter) and two air humidity levels (95% and 65% RH; ‘high RH’ and ‘low RH’) in factorial combination, yielding the four treatments cool/high RH, cool/ low RH, warm/high RH and warm/low RH. Within the chambers, three soil moisture treatments (dry, mesic and moist; i.e. around 40, 55 and 68 vol. % soil moisture) were installed, with every treatment being replicated 12-fold (2 x 2 x 3 = 12 treatments with 12 replicates, i.e. 144 pots). The pots were arranged in a randomized complete block design. Since *P. braunii* is known to occur in moderate to deep shade (Bennert 1999;

Ellenberg and Leuschner 2010), shade cloths were installed above the plants in the four climate chambers, generating a homogenous low-light environment (~40 µmol photons m^{-2} s^{-1}). The light intensity at plant height was repeatedly monitored with a quantum sensor (LI-250A; LI-Cor, Lincoln, NE, USA). The daytime period was from 7:00 h to 19:00 h. The selected temperature and RH values were derived from data measured in the field in a *P. braunii* population in the Black Forest (southwest Germany), where the local microclimate was recorded with iButton loggers (MAXIM, Munich, Germany) between 1 May and 30 September 2012. The recorded temperature (mean ± SD) in this period was 14.7 ± 4.1 °C during the day and 12.1 ± 3.4 °C at night, while mean RH (± SD) was 91.5 ± 10.1% during the day and 97.9 ± 3.5% at night. Thus, we chose 15 °C as target temperature in the experiment's current temperature treatment ('cool') and 19 °C in the warm treatment in order to investigate the species' response to a warmer summer. Temperature was reduced by 5 °C during night in both temperature treatments. The air humidity of 95% and 65% was chosen to examine the species' response to a possible drier atmosphere in the course of climate warming or after microclimate changes due to forest management activities. Microclimatic conditions in the four chambers were constantly monitored with iButton loggers. The three soil moisture categories were chosen according to field data on precipitation characterizing either a relatively dry climate (50 ml water added day^{-1} pot^{-1}) as is present at *P. braunii* sites in Saxony (east Germany) where the species went extinct in the last century (Jessen 2009), a mesic climate (80 ml day^{-1} pot^{-1}) simulating the precipitation climate in the Black Forest with stable or declining *P. braunii* populations, and a moist climate (110 ml day^{-1} pot^{-1}) resembling the climate in the Allgäu Alps(Bavaria) with a large healthy population. We decided to add defined amounts of water to all pots in the three moisture treatments instead of adding variable water amounts to maintain fixed target values of soil moisture; the latter procedure has the disadvantage that larger individuals with higher water consumption are rewarded. The resulting soil water contents were checked gravimetrically several times during the experiment. The pots were regularly rearranged in the climate chambers in a random manner to exclude the possible influence of climate gradients in the chambers.

Measured traits

Six morphological characteristics, five biomass-related traits and one physiological trait were monitored continuously to assess the vitality of the plants. The growth of frond length, frond width and length of the generative, spore-producing frond sections were measured using a simple measuring tape. Frond length was taken from the base of the frond stalk to the terminal leaf tip, frond width at the broadest section of the frond, and percentage fertile leaf section was determined by relating length of the sori-bearing frond section to total frond length. Daily growth rates (mm frond length growth day^{-1}) were calculated by dividing frond length increment between the start and end of the experiment by the length of the experiment. To determine the number of trichomes on the adaxial surface of the second-order pinnae, we counted the number of hairs under a dissecting microscope within ten 0.5 cm2 squares (0.7 x 0.7 cm) per plant and extrapolated the figures to 1 m^2. The frequency of non-sprouted leaf buds per plant and the number of plants exhibiting leaf deformation were also counted. Leaf conductance was measured with an AP4 leaf porometer (Delta-T, Cambridge, UK) at identical leaf sections (tagged leaflets at the middle of the blade) at around 11:00 h on every plant (each eight replicate measurements per plant). At the date of harvest in October 2013, aboveground (AGB) and belowground biomass (BGB) were separated from each other by cutting the fronds at the base of the leaf stalks, drying the samples at 75 °C for 72 h and weighing them. The roots were carefully washed under running tap water and also dried. The remains of leaf stalks from previous-year's fronds were excluded from the biomass analysis because decomposition was already underway. Buds for next year's fronds were assigned to the AGB fraction. Since leaf area was strongly correlated with the product of leaf length and leaf width ($R^2 = 0.9$), leaf area was extrapolated from the length and width data. The equation used was based on the harvest of 12 extra plants, the fronds of which were scanned with an EPSON Perfections V700 Photo scanner (Nagano, Japan) and analyzed with the software WinFolia version 2014a. Specific leaf area (SLA) was then calculated by dividing leaf area by leaf dry mass. Root–shoot ratio was calculated as the quotient of BGB and AGB.

Statistical analyses

All statistical analyses, except for principal components analysis (PCA), were carried out with SAS software version 9.4 (SAS Institute, Cary, NC, USA). All data were tested for normal distribution with the Shapiro-Wilk test. Non-normally distributed data, as occurred in some traits, were log-transformed. A three-factorial ANOVA was performed to quantify the effects of temperature, air humidity (RH) and soil moisture on the morphological and physiological variables. The trait 'percentage of ferns exhibiting deformation' could not be analyzed with ANOVA as it refers to non-metric data. It was, however, included in the PCA. Scheffé's test was used to detect significant differences between means of all 12 treatments (excluding the parameter 'percentage of ferns exhibiting deformations'). A significance level of $P < 0.05$ was used throughout. The PCA analyses were carried out with the software CANOCO for Windows version 4.5 (Biometris, Plant Research International, Wageningen, the Netherlands) for traits that were independent or mutually correlated with correlation coefficients ≤ 0.7.

Results

Microclimate and soil moisture conditions in the treatments

The temperature and air humidity conditions in the different treatments averaged over the duration of the experiment are given in Table 2.1. . The recorded soil moisture conditions in the dry, mesic and moist treatments were more variable across the 12 pots of a treatment than other environmental variables because plants of different sizes consumed different amounts of soil water. Soil moisture ranged from 41.8 to 42.2 vol. % in the dry treatment, 48.5–60.5 vol. % in the mesic treatment, and 66.1–70.0 vol. % in the moist treatment.

Table 2.1. Microclimatic and soil moisture conditions in the 12 treatments (A - cool/moist air, B – cool/dry air, C – warm/moist air, D – warm/dry air). Given are daily averages, or daytime and nighttime means of air temperature, relative air humidity and vapor pressure deficit (VPD), average soil water content (vol. %) in the dry, mesic and moist treatments and light intensity at plant height (PPFD).

	treatment			
	A	B	C	D
PPFD (µmol photons m^{-2} s^{-1})				
day	~ 40	~ 40	~ 40	~ 40
air temperature (°C)				
day	14.9 (± 0.05)	15.2 (± 0.1)	19.1 (± 0.1)	19.1 (± 0.04)
night	10.3 (± 0.1)	10.6 (± 0.1)	14.7 (± 0.04)	14.7 (± 0.04)
daily average	12.6 (± 0.1)	12.9 (± 0.1)	16.9 (± 0.1)	16.9 (± 0.0)
relative air humidity (%)				
day	95.3 (± 0.1)	61.1 (± 0.2)	95.9 (± 0.1)	64.4 (± 0.1)
night	96.8 (± 0.4)	63.8 (± 0.2)	96.8 (± 0.4)	64.4 (± 0.1)
daily average	96.1 (± 0.3)	62.5 (± 0.2)	96.4 (± 0.3)	64.4 (± 0.1)
VPD (Pa)				
day	80	663	90	782
night	39	475	55	609
daily average	60	569	72	696
soil moisture (%)				
dry	41.9 (± 1.9)	40.8 (± 2.0)	42.2 (± 1.6)	40.8 (± 0.9)
mesic	60.5 (± 1.9)	48.5 (± 2.2)	57.0 (± 2.2)	54.9 (± 7.3)
moist	66.1 (± 4.7)	68.9 (± 1.3)	70.0 (± 3.1)	68.9 (± 5.1)

Table 2.2. Summary of results of three-factorial ANOVAs on effects of the independent variables temperature, relative air humidty and soil moisture on 11 morphological, productivity-related or physiological traits in *P. braunii* (sum of squares, F-value, *P*-value).

Dependent variable	Temperature			Air humidity			Soil moisture		
	SS%	F	*P*	SS%	F	*P*	SS%	F	*P*
Frond length growth (mm d^{-1})	7.9	2.38	0.13	53.78	16.16	**<0.001**	12.82	1.9	0.15
Frond width growth (mmd d^{-1})	7.8	2.12	0.15	44.67	12.15	**<0.001**	18.32	2.49	0.09
Relative length of fertile frond section (%)	13.79	3.4	0.07	31.81	7.84	**<0.01**	2.97	0.37	0.69
Frequency of trichomes (n cm^{-2})	4.87	1.18	0.28	50.08	12.13	**<0.001**	22.61	2.74	0.07
Frequency of non-sprouted frond buds (%)	6.47	1.94	0.17	63.39	18.96	**<0.001**	5.79	0.87	0.42
Leaf conductance (mmol m^{-2} s^{-1})	54.07	177.41	**<0.001**	45.93	150.7	**<0.001**	0	1.21	0.30
AGB production (g $plant^{-1}$)	0.65	0.22	0.64	68.48	23.06	**<.0001**	7.62	2.56	0.08
BGB production (g $plant^{-1}$)	0.59	0.20	0.65	55.64	18.9	**<0.001**	9.96	1.69	0.19
Total biomass production (g $plant^{-1}$)	0.58	0.22	0.64	61.23	23.06	**<0.001**	13.62	2.56	0.08
Root-shoot ratio	50.25	9.09	**<0.01**	0.01	0	0.96	8.17	0.74	0.48
Specific leaf area (cm^2 g^{-1})	62.49	27.09	**<0.001**	0.81	0.35	0.56	18.04	3.91	**<0.05**

AGB = above-ground biomass, BGB = below-ground biomass. Signifcant effects are printed in bold.

Effects of air humidity, temperature and soil moisture on vigour of P. braunii

Analysis of variance showed that air humidity (RH) was the dominant environmental factor determining the morphology and productivity of *P. braunii* (Table 2.2.). All morphological traits (except SLA) and productivity-related parameters (except root–shoot ratio) and the physiological trait (leaf conductance) were influenced by RH, while air temperature only affected leaf conductance, root–shoot ratio and SLA, and soil moisture only SLA (Fig. 2.1a). Productivity was neither affected by the 4 °C temperature increase from the cool to the warm treatment, nor by the soil moisture decrease from the moist to the dry treatment according to the ANOVA. Ferns grown under high RH (95%) produced significantly longer and wider fronds as compared to ferns exposed to low RH (65%; Fig. 2.2a, for frond width growth see Table A 2.1. in the appendix). High RH increased not only frond length and width growth but also root and rhizome growth (Fig. 2.2c), and it promoted the formation of trichomes on the adaxial frond surfaces (Fig. 2.1e). Dry air reduced not only leaf conductance (Fig. 2.1f) measured close to noon (11:00 h), but also increased the frequency of plants with non-sprouted frond buds and ferns exhibiting leaf deformities (Fig. 2.1b, c); low RH also decreased the length of fertile frond sections (Fig. 2.1d). The air humidity effect on productivity parameters tended to be larger in dry soil than in mesic or moist soil (see frond length growth and ABG production; Fig. 2.2a, b). Differences between high and low RH treatments were significant according to Scheffé’s test only in a minority of cases, however. Most pronounced differences existed between the warm/high RH and the warm/low RH treatments. The strongest RH effect was detected on sprouting of the frond buds (Fig. 2.1b, Table 2.2). Except for SLA, temperature influenced none of the morphological traits significantly. However, in the warmer treatments, the length of fertile frond sections was generally smaller than in the corresponding cooler treatments (Fig. 2.1d). Temperature affected productivity only in interaction with air humidity (Table 2.3.). An air humidity-independent soil moisture effect was most visible in the case of trichome frequency, which was marginally influenced by soil moisture (P = 0.07) and was generally higher in the mesic and moist treatments. The reduced increment of frond length and width and the decreased fertility at lower RH only appeared to be significant in dry soil (Figs 2.2a, 1d). ANOVA confirmed significant interactions between RH and soil moisture for the percentage of fertile leaf sections and frond width growth (Table 2.3.). Leaf area extension decreased significantly only if low RH was associated with a dry soil. Beside the strong effect of RH on plant morphology, ANOVA revealed a significant influence of RH on biomass production (Table 2.2.). Scheffé's test indicated significantly higher AGB, BGB and total biomass production under high RH (Fig. 2.2b–d). The air humidity effect was larger on productivity than on morphological traits, and it was also visible in belowground productivity (Table 2.2.), while the soil moisture effect was not significant. The lowest biomass production was found in the warm/low RH treatment (Fig. 2.2d). In addition, temperature significantly affected the root–shoot biomass ratio, indicating that root growth decreased to a greater extent in the cooler environment than shoot growth, even though the temperature influence on productivity was not significant in the ANOVA (Table 2.2.). This may relate to the large temperature effect on SLA, with thicker fronds produced at 15 °C (21.4–29.8 cm^2 g^1) as compared to 19 °C (29.3–33.6 cm^2 g^1; Fig. 2.1a). SLA was also the only trait significantly influenced by soil moisture. Leaf conductance decreased significantly with the temperature decrease and also with the reduction in air humidity, resulting in lowest conductances under cool and low RH conditions (Fig. 2.1f). We detected no effect of soil moisture on leaf conductance. The PCA results support the results of the ANOVA (Table 2.4.). Seven of ten traits correlated with the negative section of axis one (eigenvalue = 0.77), i.e. with high relative air humidity (Table 2.4., Fig. 2.3.). The strongest negative association with axis one was found for trichome number (loading: -0.96) and total productivity (-0.87), while the strongest positive association with this axis, i.e. with low RH, was for the percentage of ferns exhibiting leaf deformities (0.94). SLA (-0.90), leaf conductance (-0.67) and root–shoot biomass ratio (-0.60) were associated with axis two (eigenvalue = 0.12), reflecting elevated temperature conditions. In contrast, aboveground productivity (0.67) and the frequency of non-sprouted buds (0.62) were associated with the positive section of axis two, i.e. reduced temperature. Thus, AGB tended to be reduced under higher temperature, in particular under low RH (cf. Fig. 2.2b). The PCA did not indicate clear correlations between the analyzed traits and the soil moisture treatment.

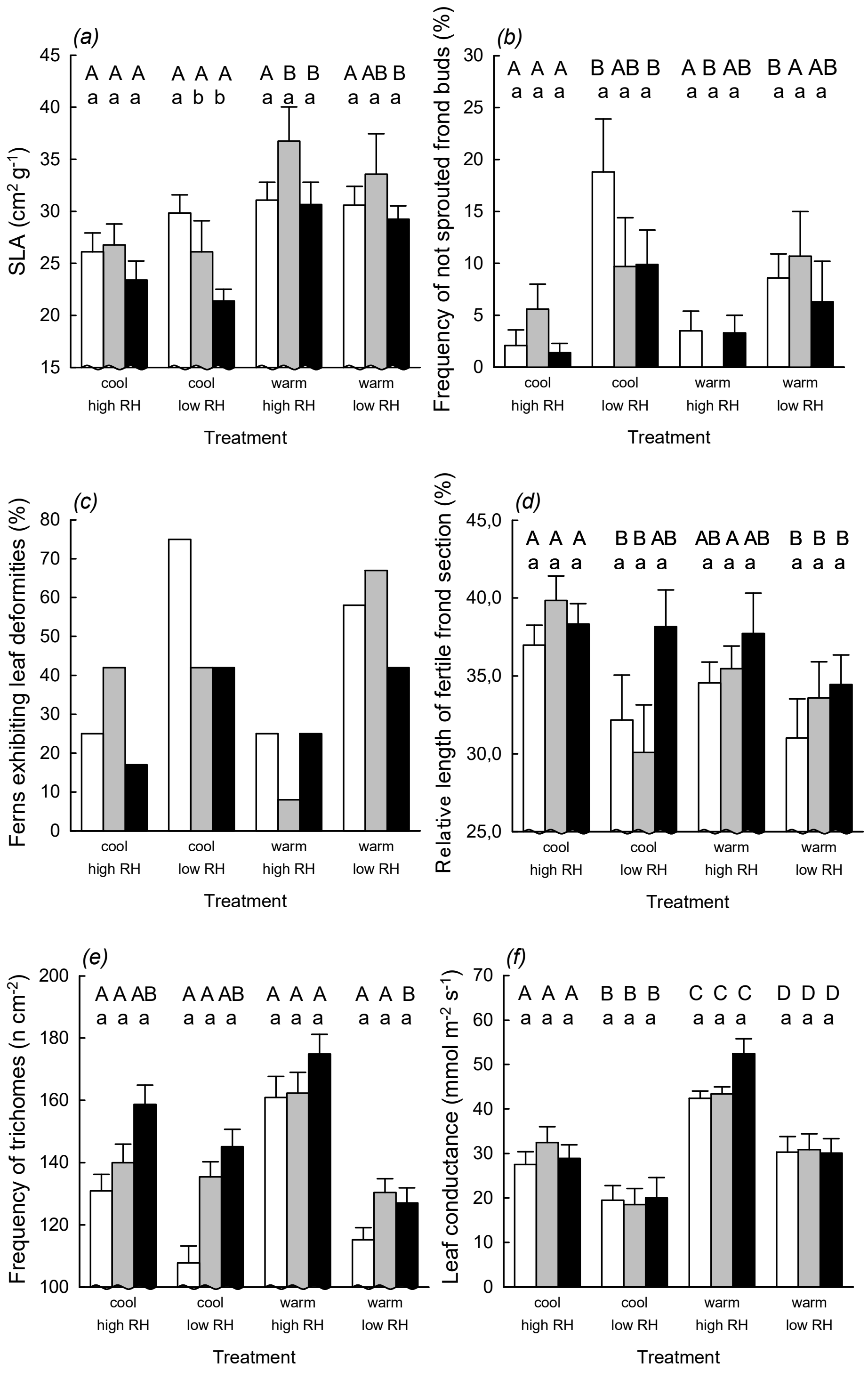

Figure 2.1. Means and SE of seven morphological and productivity-related parameters of *P. braunii* in the 12 treatments (n = 12 plants per treatment). Open bars= dry, grey = mesic, and black bars = moist soil treatment. Lowercase and capital letters indicate significant differences ($P < 0.05$) within (a, b, c) and between (A, B, C) the temperature and humidity treatments on the x-axis. SLA, specific leaf area.

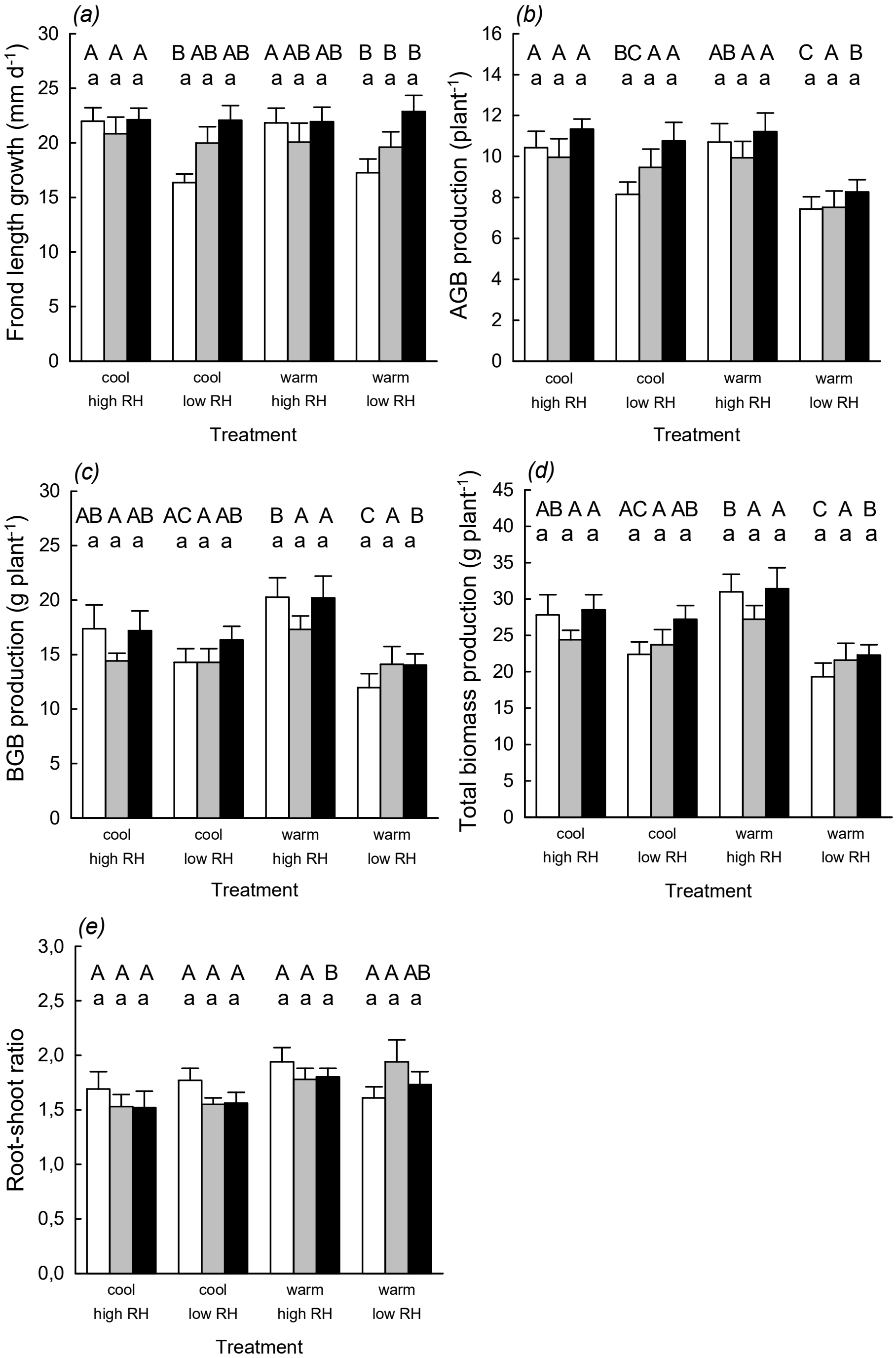

Figure 2.2. Frond length growth (a), aboveground biomass production (b), belowground biomass production (c), total biomass production (d), root – shoot ratio (e) of *P. braunii* in the 12 treatments (n = 12 plants per treatment). Bars ± SE are means of 12 individuals. Open bars = dry, grey = mesic, and black = moist soil treatment. Lowercase and capital letters indicate significant differences ($P < 0.05$) within (a, b, c) and between (A, B, C) temperature and humidity treatments on the x-axis.

Table 2.3. Summary of three-factorial ANOVAs: interaction effects of the independent variables temperature (T), relative air humidity (RH) and soil moisture (SM) on 11 morpholocical, productivity-related or physiological traits in *P. braunii* (sum of squares, F-value, P-value).

Variable	T x RH			T x SM			RH x SM			T x RH x SM		
	SS %	*F*	*P*	SS %	*F*	*P*	SS %	*F*	*P*	SS %	*F*	*P*
frond length growth (mm d^{-1})	1.12	0.34	0.56	8.23	1.24	0.29	13.27	1.99	0.14	2.88	0.43	0.65
frond width growth (mmd d^{-1})	2.8	0.75	0.39	0.74	0.1	0.90	25.31	3.45	**<0.05**	0.37	0.05	0.95
relative length of fertile frond section (%)	9.68	2.39	0.13	9.68	1.19	0.31	24.88	3.07	**<0.05**	7.18	0.89	0.41
frequency of trichomes (n cm^{-2})	12.22	2.96	0.09	4.28	0.52	0.60	3.12	0.38	0.69	2.82	0.05	0.95
frequency of non-sprouted frond buds (%)	3.54	1.06	0.31	3.23	0.48	0.62	4.34	0.65	0.52	13.23	1.98	0.14
stomatal conductance (mmol m^{-2} s^{-1})	0.00	0.27	0.60	0.00	0.62	0.54	0.00	1.1	0.33	0.00	2.98	0.05
AGB production (g plant^{-1})	19.04	6.41	**<0.05**	0.32	0.11	0.90	3.68	1.24	0.29	0.21	0.07	0.93
BGB production (g plant^{-1})	22.72	7.72	**<0.01**	1.02	0.17	0.84	8.70	1.48	0.23	1.37	0.23	0.79
total biomass production (g plant^{-1})	17.03	6.41	**<0.01**	0.58	0.11	0.90	6.58	1.24	0.29	0.38	0.07	0.93
root-shoot ratio	10.77	1.95	0.17	13.42	1.21	0.30	6.62	0.6	0.55	10.75	0.97	0.38
SLA (cm^{2} g^{-1})	0.81	0.35	0.56	9.82	2.13	0.12	5.77	1.25	0.29	2.27	0.49	0.61

AGB = above-ground biomass, BGB = below-ground biomass, TB = total biomass.Significant terms are in bold.

Table 2.4. Results of PCA on the interrelationship of ten morphological, growth-related or physiological traits in the growth traits with *P. braunii*. Given are eigenvalues, loadings of all traits that correlated to other traits with correlation coefficients ≤ 0.7 and their cumulative fit (R^2).

axes (eigenvalues)	1 (0.77)	2 (0.12)	3 (0.08)	4 (0.02)
frond length growth (mm d^{-1})	**-0.68** (0.46)	0.34 (0.57)	-0.08 (0.58)	0.04 (0.58)
relative length of fertile frond section (%)	**-0.74** (0.55)	0.10 (0.56)	0.01 (0.56)	-0.18 (0.59)
frequency of trichomes (n cm^{-2})	**-0.96** (0.92)	-0.06 (0.92)	-0.28 (0.99)	0.02 (0.10)
frequency of non-sprouted frond buds (%)	0.48 (0.26)	**0.62** (0.61)	-0.46 (0.82)	0.38 (0.96)
ferns exhibiting leaf deformities (%)	**0.94** (0.89)	-0.14 (0.91)	-0.29 (0.99)	-0.07 (0.10)
stomatal conductance (mmol m^{-2} s^{-1})	**-0.70** (0.48)	**-0.67** (0.93)	-0.10 (0.94)	-0.03 (0.94)
AGB production (g $plant^{-1}$)	**-0.65** (0.43)	**0.67** (0.87)	-0.14 (0.89)	-0.31 (0.99)
total biomass production (g $plant^{-1}$)	**-0.87** (0.76)	0.18 (0.79)	-0.21 (0.83)	-0.21 (0.88)
root-shoot ratio	-0.03 (0.00)	**-0.60** (0.37)	-0.18 (0.40)	0.00 (0.40)
SLA (cm^2 g^{-1})	-0.05 (0.00)	**-0.90** (0.82)	0.15 (0.84)	0.19 (0.88)

AGB = above-ground biomass, TB = total biomass.

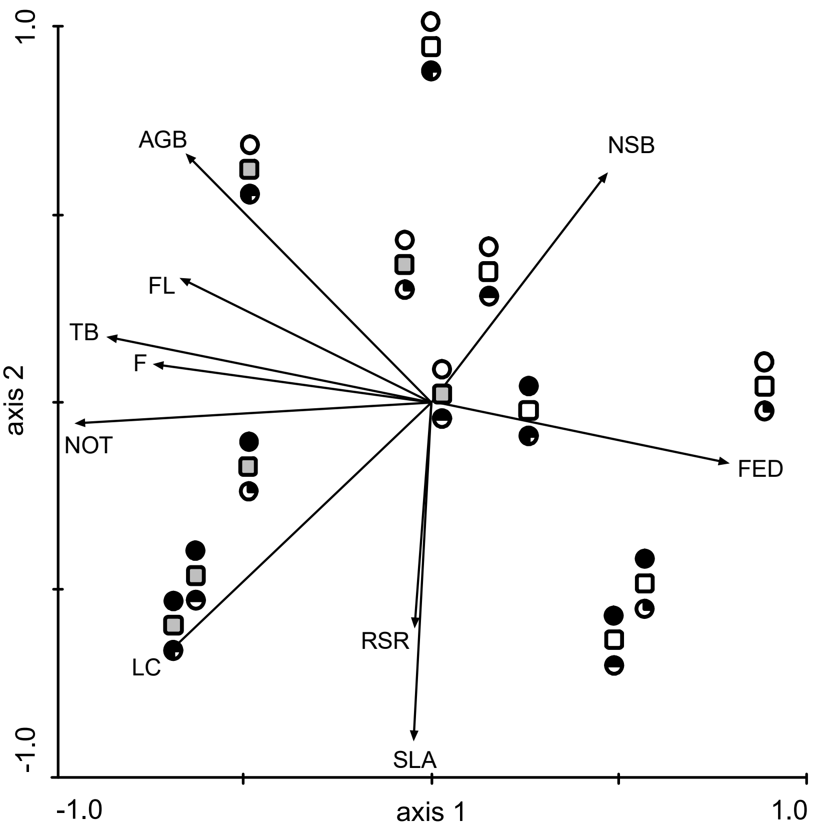

Figure 2.3. First and second axes of PCA ordination for ten morphological or productivity-related traits of *P. braunii* in the 12 treatments. Filled circles = warm, open circles = cool; grey squares = high RH, open squares = low RH; quarter-filled circles = dry, half-filled circles = mesic soil moisture, three-quarter- filled circles = moist. FL, frond length growth; F, percentage fertile frond section; NOT, number of trichomes cm^{-2}; NSB, percentage non sprouted buds, FED, percentage ferns with leaf deformities; LC, leaf conductance; AGB, aboveground biomass; TB, total biomass; RSR, root–shoot ratio; SLA, specific leaf area. The inter-correlation (r) between these variables was always ≤ 0.7.

Discussion

Air humidity as a key site factor

Nine out of 11 investigated morphological, physiological or growth-related traits of *P. braunii* were significantly affected by relative air humidity, while only one (SLA) was affected by soil moisture, and three traits by temperature (leaf conductance, root–shoot ratio, SLA; Table 2.2.). The RH effect on productivity was strong only in the warm treatments, where the plants produced around 25% more above- and belowground biomass in the high RH compared to the low RH treatment. When assessing the relative importance of environmental factors for the vigor of plants, the chosen parameter range is of crucial importance. No doubt, a larger temperature range than the 4 K investigated here (15 °C versus 19 °C) would have indicated increased importance of temperature. We defined the temperature, soil moisture and air humidity ranges in the experiment using microclimate measurements at three *P. braunii* sites in Germany, and extrapolated these to the predicted thermal and hygric conditions in a future climate with warmer and drier summers. A 4 K summer temperature increase is a value frequently predicted in regional climate change scenarios for the end of the 21st century (Beniston 2004, IPCC 2013), while the reduction in soil and air moisture of ~55% or ~30% from the moist to the dry treatment simulates microclimate changes associated with summer heatwaves, which are predicted to be more frequent in Europe in the future (Schär et al. 2004). Thus, our experiment is based on fairly realistic microclimatic and hydrologic changes likely experienced by some of the existing *P. braunii* populations in Central Europe within this century. The key role predicted in our experiment for RH is not surprising when the results of air humidity manipulation experiments with forest floor angiosperms are reviewed. When relative air humidity was reduced from 85% to 40% in a climate chamber experiment with oxygenated hydroculture (soil water potential close to 0 MPa), the temperate woodland herbs *Mercurialis perennis* and *Stachys sylvatica* reduced productivity by around 40% (Lendzion and Leuschner 2009). Correspondingly, an in situ experiment with artificial drying of the air by ~15% on the forest floor reduced productivity by around 25%. Marked air humidity effects on growth and morphology were also found for another eight temperate woodland herbs and grasses investigated in a second climate chamber study with hydroponic culture (Leuschner 2002). In a limestone beech forest, Leuschner and Lendzion (2009) found a positive association of herb layer cover not only with higher soil moisture but also with higher air humidity, supporting the experimental data from climate chambers and open-top chambers in the field. Not only herbs and grasses have been investigated in air humidity manipulation experiments but also tree saplings. Young beech plants (Fagus sylvatica) grown in moist soil in climate chambers reduced productivity by 68% when the vapor pressure deficit (VPD) was increased from 350 to 1400 Pa. A corresponding experiment with open-top chambers on the forest floor, in which air humidity was reduced by 15%, led to a productivity reduction of around 30% (Lendzion and Leuschner 2008). Conifer seedlings were also found to be sensitive to elevated VPD in their physiology and growth (Marsden et al. 1996; Darlington et al. 1997). These experiments with woody and non-woody angiosperms provide convincing evidence that air humidity acts as a soil water-independent factor on forest floor plants of temperate broadleaf forests. Elevated VPD levels seem to induce drought stress in plants, reducing growth even at ample soil moisture supply. Since *P. braunii* and other ferns grow in a similar environment to these angiosperms, we expected that their morphogenesis and growth would also be sensitive to reduced air humidity. This matches the interpretation of observational data from the field that *P. braunii* seems to have the highest requirement for elevated air humidity within its genus (Rasbach et al. 1976). As in other vascular plants, ferns possess stomata to regulate gas exchange with the atmosphere (Chater et al. 2011, Ruszala et al. 2011). However, in apparent contrast to angiosperms, fern stomata seem to act more as passive hydraulic valves, closing when leaf and guard cells dehydrate (Brodribb and McAdam 2011), while active stomatal closure, despite being proven for pteridophytes (Doi et al. 2006, Doi and Shimazaki 2008), seems to be less pronounced in this group. This may render ferns more susceptible to dehydration caused by reduced atmospheric humidity, as compared to seed plants. The latter control their leaf water balance primarily through active metabolic regulation of stomatal aperture mediated by ABA, which apparently activates ion exchange between guard cells and epidermal cells (Li et al. 2000). If this difference in leaf water loss regulation is a principal pattern distinguishing ferns from angiosperms, it points to particularly close coupling of ferns with the atmospheric moisture status. The fact that fern leaf growth is via apical meristematic cells instead of leaf basal meristems, may further enhance the dependence of these plants on VPD and air humidity: compared to seed plants, fern meristems on the tips of fronds are relatively distant from the soil water reservoir and linked to it through a relatively long flow path via roots, rhizomes and the base of the frond, while the connection to the atmospheric water pool and its evaporative demand appears to be close. These physiological and morphological characteristics should lead to a closer coupling of fern leaf water status to the atmosphere. We found a strong air humidity effect not only on productivity but also on several morphological properties of the leaves, notably increased number of trichomes on the adaxial frond epidermis, a larger relative length of fertile, sori-bearing frond sections, and a lower frequency of leaf deformities and nonsprouted frond buds. It appears that high air humidity is required by *P. braunii* for the orderly development of leaf buds and the complete unfolding of the second-order leaf pinnae, independent of a favorable soil moisture status. The number of trichomes was particularly high in the warm/high RH treatment and low in the warm/low RH treatment, but it also tended to increase with soil moisture. This pattern matches field observations in the Alps (Halloy and Mark 1996) and Costa Rica (Kluge and Kessler 2007), where the number of trichomes on leaves of seed plants and ferns was related to the humidity of the climate. Water absorption from fog (Limm and Dawson 2010) and water storage (Chie and Masahiro 2008), enhanced transpiration in damp climates or protection against excess radiation, cold and drought (Müller et al. 1981; Watkins et al. 2006) have been discussed as possible functions of leaf hairs in ferns. Tracer studies are needed to assess functions with respect to water cycling. Possible mechanisms of a dry air effect on leaf morphogenesis are the VPD control of foliar water loss, which should affect cell turgor in apical meristem, the leaf epidermis and in other leaf tissues, and resulting hydro-passive reduction in stomatal conductance, with a negative effect on CO_2 assimilation. In the absence of leaf water potential and photosynthesis measurements, our porometer measurements point to an important role of a permanent reduction in leaf conductance under lowered air humidity. In both temperature treatments, leaf conductance was reduced by about a third under 65% compared to 95% air humidity, which may have reduced photosynthesis and growth. Since we observed no SLA increase under higher RH, as may occur when only turgor-driven cell expansion is promoted, both cell water status and carbon assimilation must have profited from the more favorable atmospheric water status. As in most other traits, soil moisture did not significantly influence the leaf conductance level in the treatments, which may indicate that cuticular transpiration and its local effect on guard cell turgor is important. Plants grown in an atmosphere with vapor saturation or high RH tend to reduce the synthesis of cuticular waxes, which may increase the coupling of epidermal water status to the atmosphere. The endpoint of this development is represented in poikilohydric ferns, such as some

Hymenophyllidaceae. They possess only a very thin or no cuticle, which allows efficient water and nutrient uptake over the leaf surface but high dependence on the atmospheric moisture status (Proctor 2012).

Soil moisture and temperature effects

The minor role played by soil moisture was unexpected. Indeed, SLA was the only trait significantly influenced by soil moisture, while higher soil moisture did not promote higher growth rates. However, the data show some non-significant tendencies for better growth and higher fertility under higher soil moisture, and ANOVA revealed significant interaction between RH and soil moisture effects for two traits. In addition, in some cases, low air humidity had negative effects on productivity and plant morphology only if soil moisture was also low. This leads to the conclusion that, in some traits, high soil moisture can compensate for the negative effects of low air humidity, at least partly, but such an effect is lacking in other traits. Nevertheless, air humidity plays a more important role than soil moisture and also temperature in this species. Hence, *P. braunii* resembles more an epiphyte than a terrestrial fern (Zheng and Feng 2006). Interestingly, this finding partly disagrees with the results of Mütter et al. (1998) for Scandinavia, who investigated the distribution of *P. braunii* and found no significant association of the species' occurrences with high RH but a sigmoidal relationship with soil moisture. They describe the response of the species to air humidity as 'less clear than expected'. Most likely, the different macroclimates result in different ecological niches occupied by the species in Northern and Central Europe. While the Scandinavian populations mostly grow in deeper soils (Mütter et al. 1998), the Central European plants frequently grow on shallow rock outcrops with only thin soil layers and low water storage capacity (Bennert 1999). Hence, the dependence on high air humidity may be larger. This is indicated by the observation that the Central European populations are often found close to waterfalls or streams in foggy areas, where high air humidity might partly compensate for shallow soils. The Scandinavian and Central European populations could well represent different ecotypes adapted to contrasting temperature and moisture conditions. The warmer treatment in our experiment resulted in a significantly higher SLA of the fern fronds and a higher root–shoot ratio, i.e. promoted growth of thinner (but not larger) leaves and production of more roots per unit shoot mass. Because this species is characterized in Central Europe as a montane to alpine species with relatively low warmth requirement (Ellenberg and Leuschner 2010) and assumed susceptibility to climate warming (Brzeskiewicz and Field 2003), we had expected a more pronounced negative effect of higher temperatures on *P. braunii*. The causes of the apparent temperature sensitivity of SLA and root–shoot ratio must remain speculative. The warmer (19 °C) treatment decreased the length of the fertile frond section in both RH treatments, which might relate to altered morphogenesis of the ferns under higher temperatures. It appears that spore production is promoted by lower temperatures in the studied genotypes. However, *P. braunii* also occurs locally in the Southern Alps, where temperatures are higher. Since productivity decreased under warmer conditions only in the low air humidity treatment, but not at high RH, elevated respiration rates are not a likely explanation for this growth depression. Both warmer treatments had higher leaf conductance than the cooler treatments, suggesting that higher transpirative water loss under warmer temperatures is a more likely cause. Field measurements of Benemann (1996) confirm that *P. braunii* plants generally maintain relatively low leaf conductances that further decrease on warmer summer days due to stomatal closure.

Conclusion

To our knowledge, this study provides the first experimental evidence of a key role of air humidity in the morphogenesis and productivity of a terrestrial woodland fern, supporting our first hypothesis. *P. braunii* is an obligate air humidity hygrophyte that suffers more from a reduction in air humidity than from a decrease in soil moisture. This may require correcting our conventional soil moisture-focused perception of the environmental controls of fern water relations. Higher summer temperatures, as predicted in climate warming scenarios, however, do not seem to directly threaten this rare fern species, contradicting our second hypothesis. Nevertheless, if warming is associated with decreased atmospheric moisture, resulting from declining summer precipitation and fog abundance and/ or lowered canopy cover, substantial growth decline, decreased fertility and deformities are to be expected. With respect to the conservation of remaining populations in a warming climate, the maintenance of permanent canopy cover is essential. It is not known whether the moisture and temperature requirements of the gametophyte deviate from those of the sporophyte. The gametophytes of some tropical epiphytic ferns are highly desiccation-tolerant (Watkins et al. 2007). If this were also the case in *P. braunii*, the tiny haploid generation would probably be more susceptible to suppression from superior competitors than suffer from unfavorable air humidity. Corresponding experiments with the gametophytes are needed to answer this question.

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Appendix

Table A. 2.1. Means and standard errors (n = 12 plants) of 12 morphological, productivity-related or physiological traits in the 12 treatments. In case of leaf deformities, the percent value is given. Significant differences ($P < 0.05$) between different treatments A to D for a given soil moisture treatment are marked by different capital letters, differences between the three soil water treatments (dry, mesic, moist) within the treatments A, B, C or D, are marked by different small letters.

	Treatment A (cool / high RH)			Treatment B (cool / low RH)		
Variable	Dry	Mesic	Moist	Dry	Mesic	Moist
Frond length growth (mm d^{-1})	21.9 (± 1.2) $^{\text{A a}}$	20.8 (± 1.5) $^{\text{A a}}$	22.1 (± 1.1) $^{\text{A a}}$	16.4 (± 0.8) $^{\text{B a}}$	19.9 (±1.5) $^{\text{AB a}}$	22.1 (± 1.3) $^{\text{AB a}}$
Frond width growth (mmd d^{-1})	6.4 (± 0.4) $^{\text{A a}}$	6.0 (± 0.4) $^{\text{A a}}$	6.3 (± 0.3) $^{\text{A a}}$	5.0 (± 0.2) $^{\text{B a}}$	5.8 (± 0.5) $^{\text{AB b}}$	6.3 (± 0.3) $^{\text{A b}}$
Relative length of fertile leaf frond section (%)	37.0 (± 1.3) $^{\text{A a}}$	39.8 (± 1.6) $^{\text{A a}}$	38.3 (± 1.3) $^{\text{A a}}$	32.2 (± 2.9) $^{\text{B a}}$	30.1 (± 3.1) $^{\text{B a}}$	38.2 (± 2.4) $^{\text{AB a}}$
Frequency of trichomes (n cm^{-2})	130.9 (± 5.3) $^{\text{A a}}$	140.0 (± 5.9) $^{\text{A a}}$	158.7 (± 6.2) $^{\text{AB a}}$	107.8 (± 5.4) $^{\text{A a}}$	135.4 (± 4.9) $^{\text{A a}}$	145.1 (± 5.6) $^{\text{AB a}}$
Frequency of not sprouted frond buds (%)	1.4 (± 0.9) $^{\text{A a}}$	5.6 (± 2.4) $^{\text{A a}}$	1.4 (± 0.9) $^{\text{A a}}$	18.8 (± 5.1) $^{\text{B a}}$	9.7 (± 4.7) $^{\text{AB a}}$	9.9 (± 3.3) $^{\text{B a}}$
Ferns exhibiting leaf deformities (%)	25	42	17	75	42	42
Stomatal conductance (mmol m^{-2} s^{-1})	27.5 (± 2.9) $^{\text{AD a}}$	32.5 (± 3.6) $^{\text{AD a}}$	28.9 (± 3.0) $^{\text{AD a}}$	19.5 (± 3.2) $^{\text{B a}}$	18.5 (± 3.6) $^{\text{B a}}$	20.0 (± 4.6) $^{\text{B a}}$
AGB-production (g $plant^{-1}$)	10.4 (± 0.8) $^{\text{A a}}$	9.9 (± 0.9) $^{\text{A a}}$	11.3 (± 0.5) $^{\text{A a}}$	8.2 (± 0.6) $^{\text{BC a}}$	9.5 (± 0.9) $^{\text{A a}}$	10.8 (± 0.9) $^{\text{A a}}$
BGB-production (g $plant^{-1}$)	17.4 (± 2.2) $^{\text{AB a}}$	14.4 (± 0.72) $^{\text{A a}}$	17.2 (± 1.81) $^{\text{AB a}}$	14.3 (± 1.3) $^{\text{AC a}}$	14.3 (± 1.3) $^{\text{A a}}$	16.3 (± 1.3 $^{\text{AB a}}$
Total biomass production (g $plant^{-1}$)	27.8 (± 2.8) $^{\text{AB a}}$	24.4 (± 1.4) $^{\text{A a}}$	28.5 (± 2.1) $^{\text{A a}}$	24.4 (1.7) $^{\text{AC a}}$	23.7 (± 2.1) $^{\text{A a}}$	27.2 (± 1.9) $^{\text{AB a}}$
Root-shoot ratio	1.7 (± 0.2) $^{\text{AB a}}$	1.5 (± 0.1) $^{\text{A a}}$	1.5 (± 0.1) $^{\text{A a}}$	1.8 (± 0.1) $^{\text{AB a}}$	1.6 (± 0.1) $^{\text{A a}}$	1.6 (± 0.1) $^{\text{A a}}$
Specific leaf area (cm^2 g^{-1})	26.1 (± 1.8) $^{\text{A a}}$	26.8 (± 2.0) $^{\text{A a}}$	23.4 (± 1.8) $^{\text{A a}}$	29.8 (± 1.79) $^{\text{A a}}$	26.1 (± 3.0) $^{\text{A ab}}$	21.4 (± 1.1) $^{\text{A b}}$

	Treatment C (warm / high RH)			Treatment D (warm / low RH)		
Variable	Dry	Mesic	Moist	Dry	Mesic	Moist
Frond length growth (mm d^{-1})	21.8 (± 1.4) $^{\text{A a}}$	20.1 (± 1.7) $^{\text{AB a}}$	21.9 (± 1.3) $^{\text{AB a}}$	17.3 (± 1.3) $^{\text{B a}}$	19.6 (± 1.4) $^{\text{B a}}$	22.9 (± 1.5) $^{\text{B a}}$
Frond width growth (mm d^{-1})	6.3 (± 0.3) $^{\text{A a}}$	5.8 (± 0.5) $^{\text{AB a}}$	6.4 (± 0.4) $^{\text{A a}}$	4.8 (± 0.4) $^{\text{B a}}$	5.6 (± 0.4) $^{\text{B ab}}$	6.6 (± 0.4) $^{\text{A b}}$
Relative length of fertile leaf frond section (%)	34.6 (± 0.1) $^{\text{AB a}}$	35.5 (± 1.4) $^{\text{A a}}$	37.7 (± 2.6) $^{\text{AB a}}$	31.0 (± 2.5) $^{\text{B a}}$	33.6 (± 2.3) $^{\text{B a}}$	34.5 (± 1.9) $^{\text{B a}}$
Frequency of trichomes (n cm^{-2})	160.9 (± 3.9) $^{\text{A a}}$	162.3 (± 6.7) $^{\text{A a}}$	174.9 (± 6.3) $^{\text{A a}}$	115.2 (± 3.9) $^{\text{A a}}$	130.4 (± 4.4) $^{\text{A a}}$	127.0 (± 4.9) $^{\text{B a}}$
Frequency of not sprouted frond buds (%)	3.5 (± 1.9) $^{\text{A a}}$	0.0 (± 0.0) $^{\text{B a}}$	3.3 (± 1.7) $^{\text{AB a}}$	8.6 (± 2.3) $^{\text{B a}}$	10.7 (± 4.3) $^{\text{A a}}$	6.3 (± 3.9) $^{\text{AB a}}$
Ferns exhibiting leaf deformities (%)	25	8	25	58	67	42
Stomatal conductance (mmol m^{-2} s^{-1})	42.4 (± 1.7) $^{\text{C a}}$	43.3 (± 1.6) $^{\text{C a}}$	52.4 (± 3.4) $^{\text{C a}}$	30.4 (± 3.5) $^{\text{D a}}$	30.9 (± 3.5) $^{\text{D a}}$	30.1 (± 3.3) $^{\text{D a}}$
AGB-production (g $plant^{-1}$)	10.7 (± 0.9) $^{\text{AB a}}$	9.9 (± 0.8) $^{\text{A a}}$	11.2 (± 0.9) $^{\text{A a}}$	7.4 (± 0.6) $^{\text{C a}}$	7.5 (± 0.8) $^{\text{A a}}$	8.3 (± 0.6) $^{\text{B a}}$

BGB-production (g plant^{-1})	20.3 (± 1.8) $^{B\ a}$	17.3 (± 1.3) $^{A\ a}$	20.2 (± 1.9) $^{A\ a}$	11.9 (± 1.3) $^{C\ a}$	14.1 (± 1.7) $^{A\ a}$	14.0 (± 1.0) $^{B\ a}$
Total biomass production (g plant^{-1})	31.0 (± 2.4) $^{B\ a}$	27.2 (± 1.9) $^{A\ a}$	31.4 (± 2.9) $^{A\ a}$	19.3 (± 1.9) $^{C\ a}$	21.6 (± 2.3) $^{A\ a}$	22.3 (± 1.4) $^{B\ a}$
Root-shoot ratio	1.9 (± 0.1) $^{A\ a}$	1.8 (± 0.1) $^{A\ a}$	1.8 (± 0.1) $^{B\ a}$	1.6 (± 0.1) $^{B\ a}$	1.9 (± 0.2) $^{A\ a}$	1.7 (± 0.1) $^{AB\ a}$
Specific leaf area (cm^2 g^{-1})	31.1 (± 1.8) $^{A\ a}$	33.6 (± 3.9) $^{B\ a}$	29.3 (±1.3) $^{B\ a}$	30.6 (± 1.8) $^{A\ a}$	33.6 (± 3.9) $^{AB\ a}$	29.3 (± 1.3) $^{B\ a}$

CHAPTER 3

Vulnerability analysis of the rare and endangered woodland fern *Polystichum braunii* in Germany: three possible causes of population decline

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Abstract

Background: Various rare and endangered temperate ferns are being threatened by their recent population decline, but there is limited understanding of the causes behind it.

Aims: This study attempted to identify the possible drivers of regional population decline and extinction in the globally distributed woodland fern *Polystichum braunii*.

Methods: A comparison was undertaken of the climatic, edaphic and phytosociological characteristics of sites with increasing, decreasing or recently extinct populations in Germany.

Results: A significantly higher frequency of episodes of low relative air humidity (<60 %) was found at sites with decreasing or extinct populations compared to habitats with population increases. Sites with decreasing or extinct populations were also characterised as having less summer precipitation (<500 mm year^{-1}) and a shorter duration of snow cover (<110 days year^{-1}) than sites with increasing populations. The latter had significantly higher moss cover (56 % of the forest floor), but less cover by a tree litter layer (23 %) compared to decreasing (36 % and 38 %) or recently extinct populations (22 % and 52 %). All increasing populations were located in intact *Tilia* - *Acer* ravine forests, while those suffering population decline were mostly located in *Fagus*-dominated forests.

Conclusions: It was concluded that the probable causes of the recent decline in German *P. braunii* populations are reduced air humidity levels, decreasing snow duration or a shift from moss-covered to tree litter-covered forest floors due to climate warming or altered forest management.

Key words: climate warming, conservation, litter and moss cover, snow cover, precipitation decline, vapour pressure deficit

Introduction

In the last few decades, there has been a rapid increase in the loss of plant species in Central Europe and other temperate regions, which is thought to be primarily a consequence of agricultural intensification, widespread eutrophication and increasing dispersal limitation due to habitat fragmentation (e.g. Tilman et al. 2001; Rockström et al. 2009; Krauss et al. 2010; Meyer et al. 2013). Repeat surveys, monitoring data and consecutive assessments of red-listed species suggest that species losses are generally less pronounced in forests than in the non-forest vegetation of Central Europe (Korneck et al. 1998). The plot-level diversity of forest floor phanerogams has remained stable or even increased in many forests that have been monitored over a number of decades. However, closer inspection of the data reveals distinct shifts in community composition in many forests, with decreases in characteristic woodland phanerogams that depend on long forest continuity and low disturbance levels, and increases in generalist species that tolerate disturbances and also occur in clearings, at forest edges and in open non-forest vegetation (Lameire et al. 2000; Jantsch et al. 2013). Furthermore, major losses in the species pool have been recorded for the lichens and bryophytes over the last 50-150 years, which are thought to have been caused by the disappearance of old-growth forests, the shift to high forest management with more closed canopies, nitrogen and acid deposition, and (in the case of bryophytes) an apparent decrease in humidity due to widespread drainage that has been applied since the Middle Ages (Koperski 1998; Ellenberg and Leuschner 2010). For example, repeat inventories in north-west Germany after 100-150 years have shown decreases between 28% and 30% in the regional lichen (Hauck et al. 2013) and bryophyte species pool (Dittrich et al. 2014).

Ferns constitute an important element of the herbaceous flora of many types of woodland where they can serve as indicators of environmental conditions (e.g. Grinspoon et al. 2003; Duque et al. 2003; Nagalingum et al. 2015). While the majority of woodland ferns in Central Europe are widespread and not endangered, several taxa are rare and some even threatened by regional extinction. The causes of recent population decline of ferns are not well understood (Bennert 1999; Jessen 2009) and there is generally poor knowledge of the biology of ferns (Given 1993; Kelly 1994; Testo and Watkins 2013; Rünk et al. 2004; Watkins et al. 2007; Bucharová et al. 2010).

In Central Europe, one of the rarest woodland ferns is *Polystichum braunii* (Spenn.) Fée (Dryopteridaceae). It is present in about 15 regions, 4 of them being located in Germany (Figure 3.1.). The species occurs in the temperate and boreal zones of Eurasia and North America, but is highly patchily distributed. Most populations consist of only 10-100 individuals; populations are often separated by hundreds to thousands of kilometres (Hultén 1961; Wagner 1993; Brzeskiewicz and Fields 2003). Although the species is not vulnerable in most of its range (Appendix, global rank G5: NatureServe 2015) and is not included in the IUCN Red List of Threatened Species, many populations have recently declined and the species is therefore considered to be highly endangered in several countries. In Germany, probably only 10 populations remain today, while at least an equivalent number have become extinct in the last 50 years, including very large populations with several hundred plants (cf. Bennert 1999; Baier et al. 2005; Jessen 2009; Wildlife Conservation Programme Baden-Württemberg). About half of the remaining populations in Germany are very small, with declining numbers that could probably benefit from targeted conservation measures. Lastly, some of the German populations may represent important links between the populations in Scandinavia and those in central and southern Europe (Baier et al. 2005).

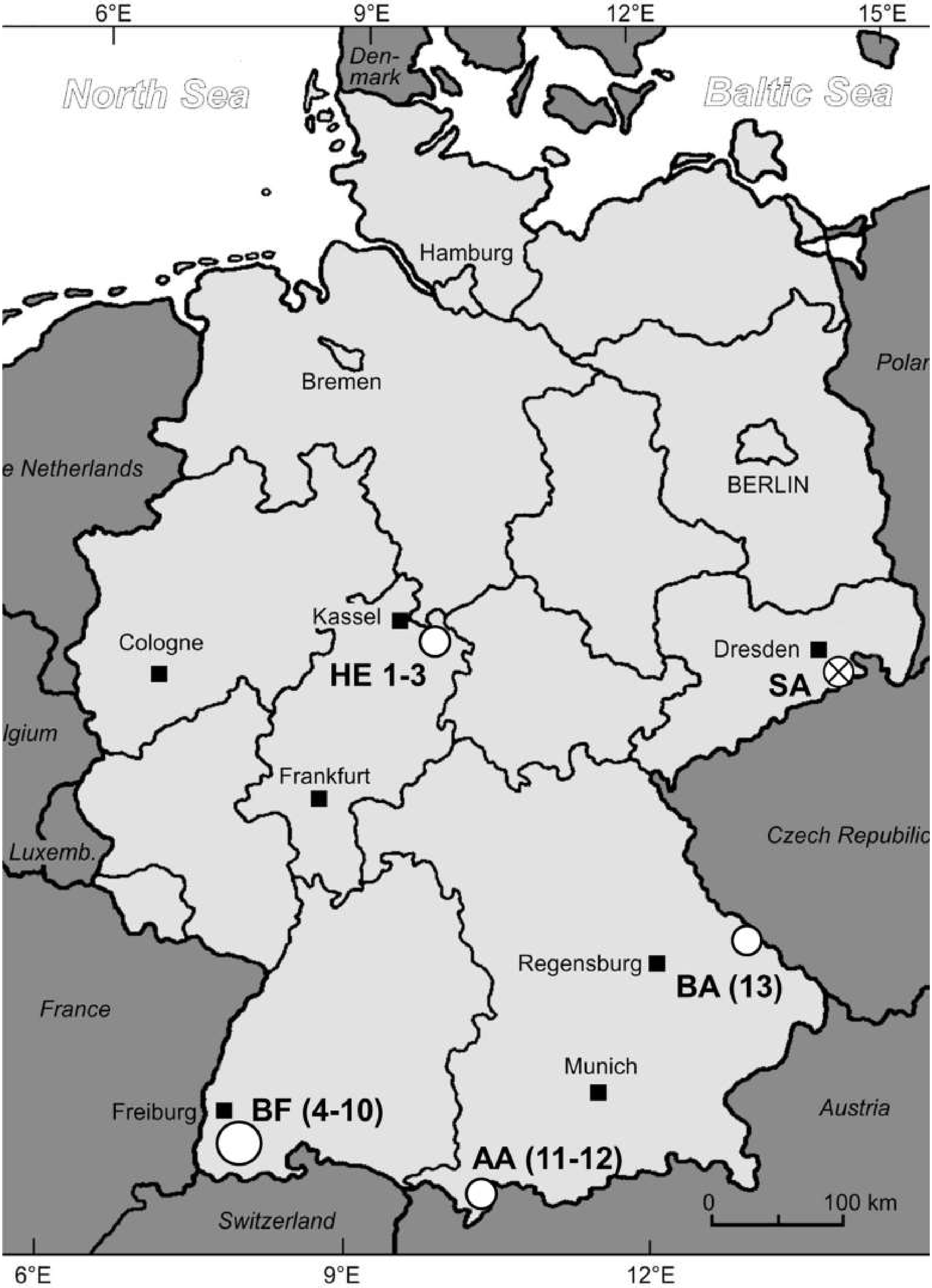
6°E
9°E
12°E
15°E
Den-
mark
North Sea
Baltic Sea
Hamburg
Bremen
Polar
e Netherlands
BERLIN
Kassel
Cologne
HE 1-3
Dresden
SA
lgium
Frankfurt
Luxemb.
Czech Republic
Regensburg
BA (13)
France
Munich
Freiburg
BF (4-10)
Austria
AA (11-12)
0
100 km
Switzerland
6°E
9°E
12°E

Figure 3.1. Locations of *P. braunii* populations with increasing, decreasing or extinct populations in Germany. Open circles = regions with occurrences of *P. braunii*; circles with cross = regions with recently extinct populations. AA, Allgäu Alps; BA, Bavarian Forest; BF, Black Forest; HE, Northern Hesse; SA, Saxon uplands. Figure 1 corresponds to Table 1 (see column "Pop. no."), which gives detailed information on the German populations.

Small population size may not negatively affect the populations' vitality since polyploids are generally well adapted to genetic bottleneck situations (cf. Ohno 1970; Gu 2003; Comai 2005). Various disturbance factors that affect populations include landslides and flooding (Horn 1992; Bennert 1999), changes in local hydrological regimes (Bennert 1999; Rasbach et al. 1976), climate warming, and the related reduction in winter snow cover (Eberle 1966; Mütter et al. 1998; Brzeskiewicz and Field 2003; Jessen 2009). Only a few studies have explicitly addressed the possible effects of climate warming on ferns (e.g. Limm and Dawson 2010; Testo and Watkins 2013; Bystriakova et al. 2014). A recent climate chamber experiment with *P. braunii* sporophytes showed that the fern is indeed highly influenced in its morphogenesis and productivity by site water relations and that air humidity is much more important than soil moisture within the relevant range of environmental conditions (Schwerbrock and Leuschner 2016). Observational studies suggest that bryophyte cover on the forest floor may also be an important factor that strongly correlates with fern species richness (Kessler 2001).

With the aim of improving our understanding of the causes driving population decline in the *P. braunii* populations of Germany, an observational study was conducted on 10 remaining populations of the species, investigating the microclimatic, edaphic and vegetation structural parameters likely to be of importance. Based on the population size data of Bennert (1999) and the authors' own successive population size counts in 2011 and 2014, populations with either increasing or decreasing recent population trends were identified (five populations each) along with a third category where populations had recently become extinct (three populations). The habitat conditions of these three categories were compared to infer possible causes underlying the contrasting population trends. Habitat comparison has been found to be a useful tool in detecting the reasons behind species decline and extinction (Bessinger and McCullough 2002; Aguraiuja et al. 2008), particularly when combined with experimental studies on the physiological requirements of the target species, as was the case here, taking into account the results from the study of Schwerbrock and Leuschner (2016). A comparative observational study across different habitats has the advantage of ensuring that the measured environmental parameters are of relevance for the entire life cycle of the ferns, i.e. they cover both the gametophyte and the sporophyte generations. From the findings of the climate chamber study, literature reports on possible threatening factors and recent trends in the regional climate, our questions were as follows: (i) Are populations with a negative trend or recent extinction found in sites with lower air humidity than increasing populations? (ii) Are the climatic conditions required by *P. braunii* being adversely affected by recent climate warming? and (iii) Does a dense bryophyte layer favour the fern's survival?

Material and methods

Study sites

The fieldwork was carried out in 13 populations of *P. braunii* in three German regions with low mountain ranges (Southern Black Forest [BF], Bavarian Forest [BA], Northern Hesse [HE]) at 500-1300 m a.s.l. and in the Allgäu Alps (Bavaria [AA]) at 1300-1400 m a.s.l. (Figure 3.1.). The sites are exposed to a temperate humid oceanic (BF, AA, HE) to humid subcontinental climate (BA) with a growing season precipitation (May-September) of 400-1000 mm. Mean annual precipitation is 900-1900 mm and mean annual temperature is ca. 5.0-6.0 °C. Mean winter and summer temperatures range between ca. -3.0 °C and 0 °C, and ca. 12.0 °C and 15.0 °C respectively (1961–1990, data provided by Deutscher Wetterdienst [DWD], Offenbach). Five locations harbour *P. braunii* populations with a decreasing population size (HE, BA and BF), five with increasing populations (BF and AA) and in another three locations the fern is locally extinct (HE and BF, see Table 3.1.). *P. braunii* occurs at these locations primarily in ravine forest communities in which *Acer pseudoplatanus* L., *Fraxinus excelsior* L., *Ulmus glabra* L. and *A. platanoides* L. constitute the tree layer. The frequent occurrence of herbaceous species such as *Impatiens noli-tangere, Circaea lutetiana,* *Chrysosplenium alternifolium* and *C. oppositifolium* and *Oxalis acetosella* indicates humid growing conditions. At montane to upper montane elevation in the Allgäu Alps, other frequently co-occurring species are *Actaea spicata* L., *Moehringia muscosa* L.*, Petasites albus* (L.) J. Gaertn and *Viola biflora* L.

Microclimatic measurements

At all 13 locations, iButton loggers (MAXIM, Munich, Germany) were installed for a one-year period (1 Sept 2012 – 1 Sept 2013) in the immediate vicinity of the extant or extinct *P. braunii* populations. The data loggers were attached to wooden stakes at a height of 80 cm above the ground, protected by plastic tubes (diameter 10.5 cm), with the upper side closed to prevent contact with snow and rainwater and direct radiation exposure. Temperature and relative air humidity (RH) were recorded every 3 h. Additional data loggers for soil temperature measurement were wrapped in plastic foil before being inserted at 10 cm soil depth in close proximity to *P. braunii* plants (also measuring every 3 h). Constant soil temperature conditions around 0 °C were used as indicators of snow cover.

Long-term precipitation and snow cover data for the period 1960-2010 were obtained from the DWD (German Meteorological Service, Offenbach). Data from meteorological stations (each 3-5) as close as possible to the study sites with roughly the same elevation were used. The data for the HE location had to be extrapolated from a lower elevation station using the precipitation data of Hauschulz (1959) for this region.

Microhabitat and soil analyses

In August 2014, litter cover (in %), litter mass (in g m^{-2}), moss cover (in %), moss layer thickness (in cm) and soil profile depth to the bedrock (in cm) were measured and the total number of fern species was recorded in 1 -m^2 plots. Eight to twenty plots per population (in function of patch size, or conservation restrictions) were sampled, half of which contained the fern. The leaf litter collected from an area of 30 cm x 30 cm was weighed after drying the samples at 75 °C for 72 h. Soil chemical analyses were carried out on the topsoil material consisting of the lower organic layer horizons (Of and Oh layers) and the uppermost mineral soil (Ah horizon) in which most of the roots were found. The pH (H_2O) was measured with a pH meter (Orion star A221, Thermo Fisher Scientific, Waltham, MA., USA) in a solution of 10 g of fresh soil suspended in 25 ml of distilled water. The exchangeable concentrations of magnesium, potassium, calcium and aluminium in the topsoil were detected by Inductively Coupled Plasma analysis (Optima 5300 DV, Perkin Elmer, Waltham, MA, USA) after extraction with a 0.2 N $BaCl_2$ solution. To estimate the content of plant-available phosphorus (P_{resin}), 1 g of soil was suspended in 30 ml of water. The phosphate was then extracted by the addition of anion-exchange resin (Dowex, 1 x 8-50) to the suspension for 16 h. Subsequently, the absorbed phosphate was exchanged with NaCl and NaOH solutions and the P concentration then determined colorimetrically through the vanadate-molybdate complex. The C_{org}:N_t ratio in the soil material was measured using a C/N element analyser (Vario EL 3, Elementar, Hanau, Germany) after drying the samples at 70 °C for 48 h. To identify the environmental conditions associated with *P. braunii*, the environmental variables were compared for plots with fern presence (n = 34-37) and absence (n = 32-34) in the five increasing populations.

Statistical analyses

All statistical analyses were carried out using the software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The Kruskal-Wallis test was used to identify significant differences between means of the categories ‘increasing’, ‘decreasing’ and ‘extinct’ populations, and for individual environmental variables associated with locations with fern presence and fern absence. Graphs were produced with the software Xact 7, SciLab (Saint-Yrieix, France).

Table 3.1. Some physiographic and population characteristics of the 13 study sites in Germany with recently increasing, decreasing or extinct populations of *P. braunii*.

					Population sizes				
Region	m a.s.l.	Forest type	Bedrock type	Protected area	1990s	2011	2014	Reproduction	Pop. no.
N Hesse, C Germany	700	Beech forest, non-assignable	Basaltic rock (s)	yes	8	5	4	no	1
N Hesse, C Germany	650	Fraxino-Aceterum pseudoplatani	Basaltic rock (s)	yes	last verification ca. 1990			no	2
N Hesse, C Germany	700	Luzulo-Fagetum	Basaltic rock (s)	yes	last verification 1987			no	3
S Black Forest, SW Germany	560	Fraxino-Aceretum pseudoplatani	Migmatic rock (s)	no	last verification 1985			no	4
S Black Forest, SW Germany	1052	Ulmo glabrae-Aceretum pseudoplatani	Migmatic rock (s)	no	N/A	9	8	no	5
S Black Forest, SW Germany	600	Tilio-Acerion	Migmatic rock (s)	yes	7	7	3	no	6
S Black Forest, SW Germany	818	Tilio-Acerion	Eclogite-amphibolite (s)	yes	12	16	20	yes	7
S Black Forest, SW Germany	550	Fraxino-Aceretum pseudoplatani	Migmatic rock (s)	yes	21	9	6	no	8
S Black Forest, SW Germany	618	Fraxino-Aceretum pseudoplatani	Gneiss (s)	yes	1	N/A	10	yes	9
S Black Forest, SW Germany	600	Fraxino-Aceretum pseudoplatani	Gneiss (s)	yes	min. 150	ca. 400	ca. 400	yes	10
Allgäu Alps, S Germany	1100	Aceri-Fagetum	Hornstein limestone (b)	yes	11	ca. 150	ca. 180	yes	11
Allgäu Alps, S Germany	1350	Aceri-Fagetum	Sandstone (s)	no	45	ca. 300	ca. 350	yes	12
Bavarian Forest, SE Germany	780	Luzulo-Fagion	Cordierite and silimanite (s)	yes	3	1	1	no	13

Notes: s: siliceous, b: basic, N/A: no information available, reproduction: identification of young sporophytes <10 cm, Pop. no.: corresponding number on the distribution map in Figure 1a.

Results

Microclimate of sites with increasing, decreasing and extinct P. braunii *populations*

Microclimate measurements 80 cm above the ground and soil temperature showed a significantly higher frequency of episodes with reduced air humidity and a shorter duration of snow cover in sites with decreasing or extinct fern populations than at locations with increasing populations (Table 3.2.). Correspondingly, mean vapour pressure deficit (VPD) levels were higher and mean RH lower in sites with decreasing or extinct populations, while air and soil temperatures were similar. Periods with RH <60 % occurred two and three times more frequently in decreasing and extinct populations respectively than in increasing populations, and those <50 % RH two and six times more frequently. VPD was higher and RH lower at extinct and decreasing populations in the growing season (April – September) but not in winter (Figure 3.2. and Figure 3.A1).

Table 3.2. Summary of the microclimatic conditions during the period 1 Sept 2012 – 1 Sept 2013 at locations with increasing, decreasing and extinct populations (n = 3-5 locations per category) of *P. braunii*.

	Population status		
Parameters	extinct	decreasing	increasing
Annual mean air temperature (°C)	6.3 (± 0.4)	6.1 (± 0.4)	6.7 (± 0.4)
Absolute minimum	-12.4	-11.0	-9.7
Absolute maximum	25.8	26.1	25.8
Mean monthly minimum	-2.1 (± 0.3)	-2.2 (± 0.5)	-1.0 (± 0.5)
Mean monthly maximum	16.3 (± 0.3)	15.0 (± 0.3)	14.9 (± 0.4)
Growing season mean (April-October)	11.3 (± 0.3)	10.7 (± 0.4)	11.5 (± 0.7)
Mean RH (%)	94.4 (± 0.8)	96.3 (± 0.9)	96.9 (± 0.4)
Absolute minimum	39.4	48.3	49.4
Absolute maximum	100.0	100.0	100.0
Mean monthly minimum	64.5 (± 1.6)	68.6 (± 1.4)	68.9 (± 2.7)
Mean monthly maximum	100.0 (± 0.0)	100.0 (± 0.0)	97.2 (± 2.8)
Growing season mean (April-October)	91.7 (± 0.7)	94.4 (± 0.9)	95.40 (± 0.6)
FDE <90 % RH	616.0 (± 95.4)^{A}	401.6 (± 100.1)^{B}	300.7 (50.6)^{C}
FDE <80 % RH	255.7 (± 58.3)^{A}	166.4 (± 59.6)^{B}	108.0 (± 22.1)^{C}
FDE <70 % RH	99.7 (± 24.7)^{A}	64.4 (± 29.2)^{B}	36.0 (± 8.7)^{C}
FDE <60 % RH	35.3 (± 11.2)^{A}	18.8 (± 8.9)^{B}	9.7 (± 2.4)^{C}
FDE <50 % RH	8.7 (± 5.2)^{A}	2.6 (± 0.8)^{B}	1.3 (± 1.3)^{B}
Mean VPD (Pa)	53.3 (± 6.5)	36.2 (± 8.7)	29.5 (± 4.3)
Growing season mean (April-October)	115.3 (± 6.9)	81.1 (± 8.7)	68.3 (± 8.1)
Mean soil temperature (°C)	6.8 (± 0.5)	6.8 (± 0.3)	6.5 (± 1.3)
Absolute minimum	0.4	0.4	0.3
Absolute maximum	16.8	18.8	16.4
Mean monthly minimum	4.5 (± 0.6)	4.1 (± 0.2)	3.8 (± 0.9)
Mean monthly maximum	9.8 (± 0.3)	10.0 (± 0.4)	9.5 (± 1.8)
Growing season mean (April-October)	9.9 (± 0.4)	9.9 (± 0.4)	10.5 (± 0.6)
Days with snow cover	84.0 (± 36.0)^{A}	110.2 (± 4.0)^{A}	136.5 (± 26.4)^{B}

Notes: Means and standard errors are given, or minimums and maximums where appropriate. FDE: frequency of measuring points with values below a given RH threshold (total = 2920 points). Different upper case capital letters indicate significant differences between categories (P <0.05)

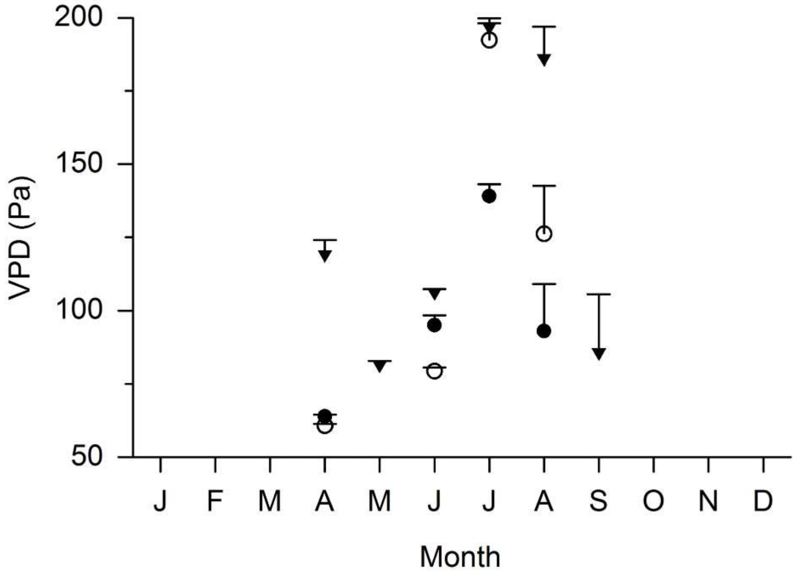

Figure 3.2. Monthly means of the atmospheric vapour pressure deficit (VPD) at 80 cm above ground at locations with increasing (filled circles), decreasing (open circles) and extinct populations (triangles) of *P. braunii* during the period 1 Sept 2012 – 1 Sept 2013. Means of each group of 3-5 sites are given with standard error.

Long-term climate records

The 50-year record of snow cover corroborates the snow cover duration patterns inferred from the present study's temperature measurements in 2012/2013 by indicating on average 110-170 days with snow cover at sites with increasing fern populations (BF, AA), 70-115 days in sites with decreasing populations (BA, HE), and only 20-50 days in the SA site with extinct populations (Figure 3.3.). In all five regions, there has been a non-significant trend of decreasing duration of snow cover in the last 50 years. Sites with increasing, decreasing and extinct populations differed with respect to growing season precipitation (May – September) and rainfall in May and June: growing season precipitation was generally above 550 mm in sites with increasing populations, but below this threshold in decreasing and extinct populations (Figure 3.4a). Rainfall in May and June, when ferns are sprouting, was below 100 and 125 mm respectively in the populations exhibiting a negative trend, but ranged between 100 and 200 mm in May and 140 and 250 mm in June at locations with increasing populations (Figure 3.4b and c). Except for the BA sites (precipitation decrease only in June), growing season precipitation and May and June rainfall had decreased in the period 1960 – 2010.

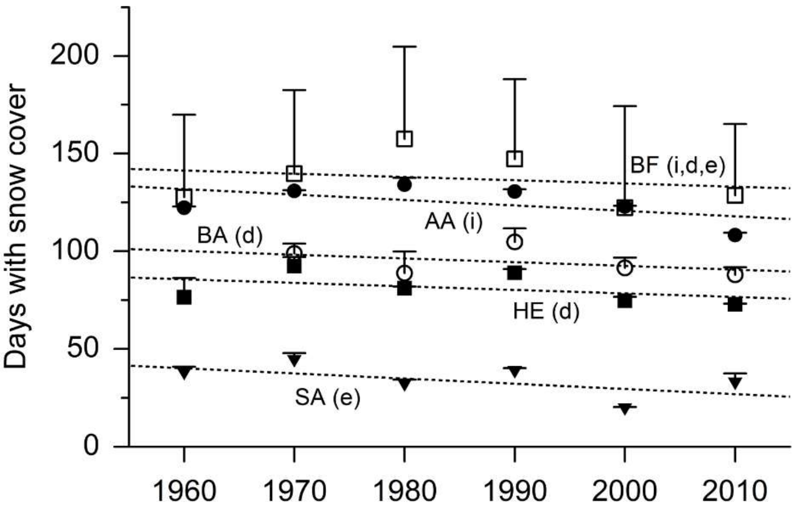

Figure 3.3. Long-term trends in mean annual days with snow cover in the period 1960 – 2010 in the four study regions with existing *P. braunii* populations (AA – Allgäu Alps, BF – Southern Black Forest, BA – Bavarian Forest, HE – Northern Hesse) and the Saxon uplands (SA) with only recently extinct populations. Each region contained four to eight populations of increasing (i), decreasing (d) or extinct (e) status. Decade-long precipitation means (± standard error) are given preceding the indicated year from three to five meteorological stations in the vicinity of the populations. Linear regression equations, *P*-values (first number) and R^2 (second number) for the five areas are as follows: AA: y -0.279x+678.64, 0.13, 0.31: BF: y = -0.1666x+467.78, 0.33, 0.05: BA: y = -0.19x+472.48, 0.24, 0.17: HE: y = -0.1806x+439.5, 0.20, 0.18 and SA: y = -0.2631x+555.86, 0.11, 0.17

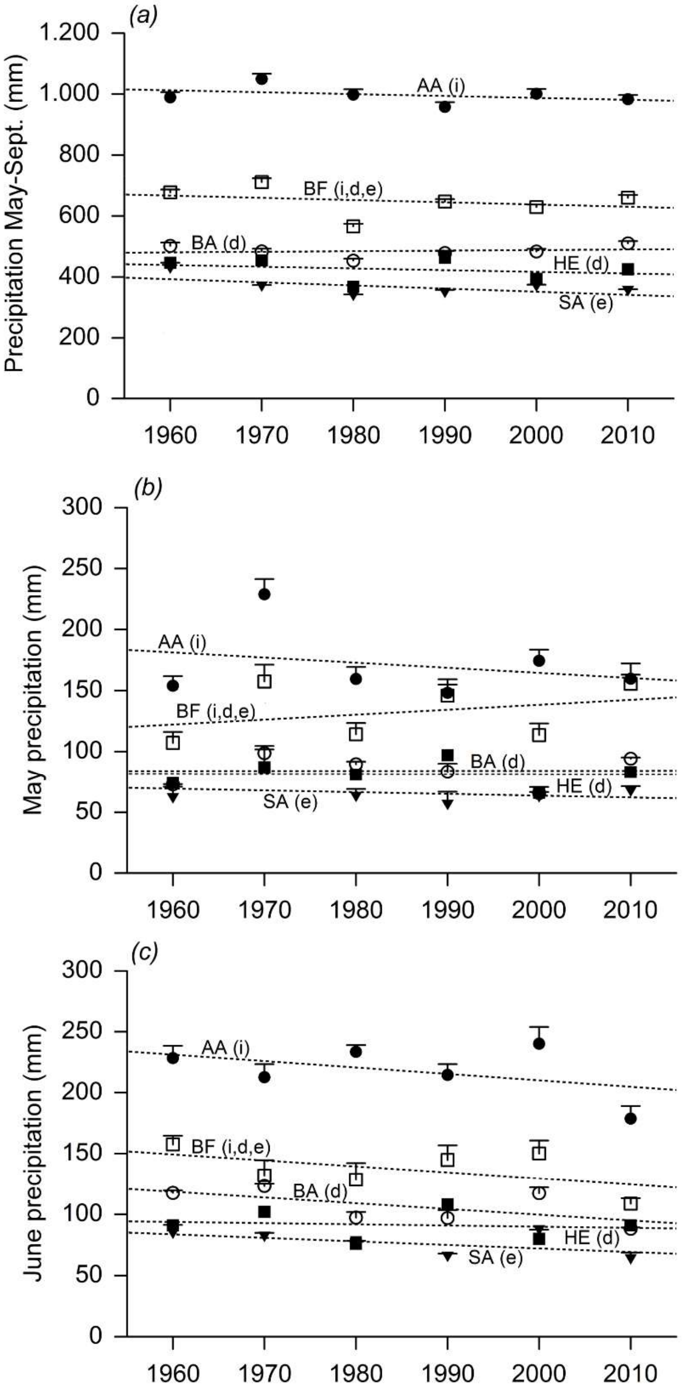
(a)
Precipitation May-Sept. (mm)
1.200
1.000
800
600
400
200
0
AA (i)
BF (i,d,e)
BA (d)
HE (d)
SA (e)
1960
1970
1980
1990
2000
2010
(b)
May precipitation (mm)
300
250
200
150
100
50
0
AA (i)
BF (i,d,e)
BA (d)
HE (d)
SA (e)
1960
1970
1980
1990
2000
2010
(c)
June precipitation (mm)
300
250
200
150
100
50
0
AA (i)
BF (i,d,e)
BA (d)
HE (d)
SA (e)
1960
1970
1980
1990
2000
2010

Figure 3.4. Long-term trends in summer precipitation *(a)*, May precipitation *(b)* and June precipitation *(c)* in the period 1960 – 2010 in the four study regions with existing *P. braunii* populations (AA – Allgäu Alps, BF – Southern Black Forest, BA – Bavarian Forest, HE – northern Hesse) and the Saxon uplands (SA) with only recently extinct populations. Each region contains four to eight populations with increasing (i), decreasing (d) or extinct (e) status. Decade-long precipitation means (± standard error) preceding the indicated year are given from three to five meteorological stations in the vicinity of the populations. Linear regression equations, *P*-values (first number) and R^2 (second number) for the five areas are as follows: AA: $y = 0.621x+2229.5$, 0.23, 0.15: BF: $y = -0.7273x+2092.4$, 0.30, 0.08: BA: $y = 0.1865x+115.15$, 0.37, 0.03: HE: $y = -0.5439x+1504.7$, 0.30, 0.08: SA: $y = -1.024x+2399.7$, 0.10, 0.37 (a) : AA: $y = -0.4182x+1000.8$, 0,31, 0.07: BF: $y = 0.404x-669.7$, 0.26, 0.11: BA: $y = 0.0044x+75.42$, 0.49, 0.00: HE: $y = -0.0129x+107.2$, 0.49, 0.00: SA: $y = -0.1436x+350.82$, 0.31, 0.07 (b) : AA: $y = -0.5265x+1263.1$, 0.19, 0.20: BF: $y = -0.4955x+1120.4$, 0.14, 0.28: BA: $y = -0.4699x+1039.5$, 0.10, 0.38: HE: $y = -0.097x+283.83$, 0.39, 0.02: SA: $y = -0.2887x+649.53$, 0.13, 0.29 (c)

The structure of moss and litter layers and soil chemistry

In sites with increasing fern populations, the cover (56.0 %) and height (3.7 cm) of the moss layer were significantly greater than at sites with decreasing (35.9 % and 3.6 cm) or extinct populations (22.4 % and 1.5 cm, Figure 3.5a and b, Table 3.3.). Moss cover was also significantly higher at sites with the presence of *P. braunii* than at nearby locations where the fern was absent (79.7 % vs. 30.3 %); the moss layer was also thicker at the former sites (4.5 vs. 2.9 cm, differences significant; Figure 3.6a and b and Table 3.4.). The opposite was found for litter cover and litter mass. Sites with increasing fern populations had less litter cover (23.1 %) and less litter mass (89.6 g m^{-2}) than sites with decreasing (38.1 %, 152.8 g m^{-2}) or extinct populations (52.3 %, 208.4 g m^{-2}; Figure 3.5b, Table 3.3.). Correspondingly, sites with the presence of *P.* were characterised by significantly less litter cover and mass (10.4 % and 56.9 g m^{-2}) than nearby sites without the fern (36.9 %, 123.8 g m^{-2}; Figure 3.6b and Table 3.4.).

Sites with increasing fern populations also seemed to differ in the chemistry of the topsoil (organic Of and Oh horizons and uppermost mineral soil Ah horizon) from sites with decreasing or extinct populations. Where fern populations were increasing, the C:N ratio was lower, and the cation-exchange capacity (CEC) and the ratio of exchangeable Ca to Al in the topsoil higher than at sites with decreasing or extinct populations (Table 3.3.); pH and the N_t and P_{resin} concentrations showed no directional trends. The topsoil concentration of exchangeable Al was at least twice as high in sites with decreasing or extinct populations (36.4 and 44.4 µmol g^{-1}) than in those with increasing populations (17.0 µmol g^{-1}, Figure 5d; difference significant between increasing and extinct populations). Apart from the thicker moss and thinner litter layer, sites with the presence of *P. braunii* only slightly differed in soil chemistry from sites without the fern (somewhat higher N_t, P_{resin} and CEC in the topsoil, Table 3.4.).

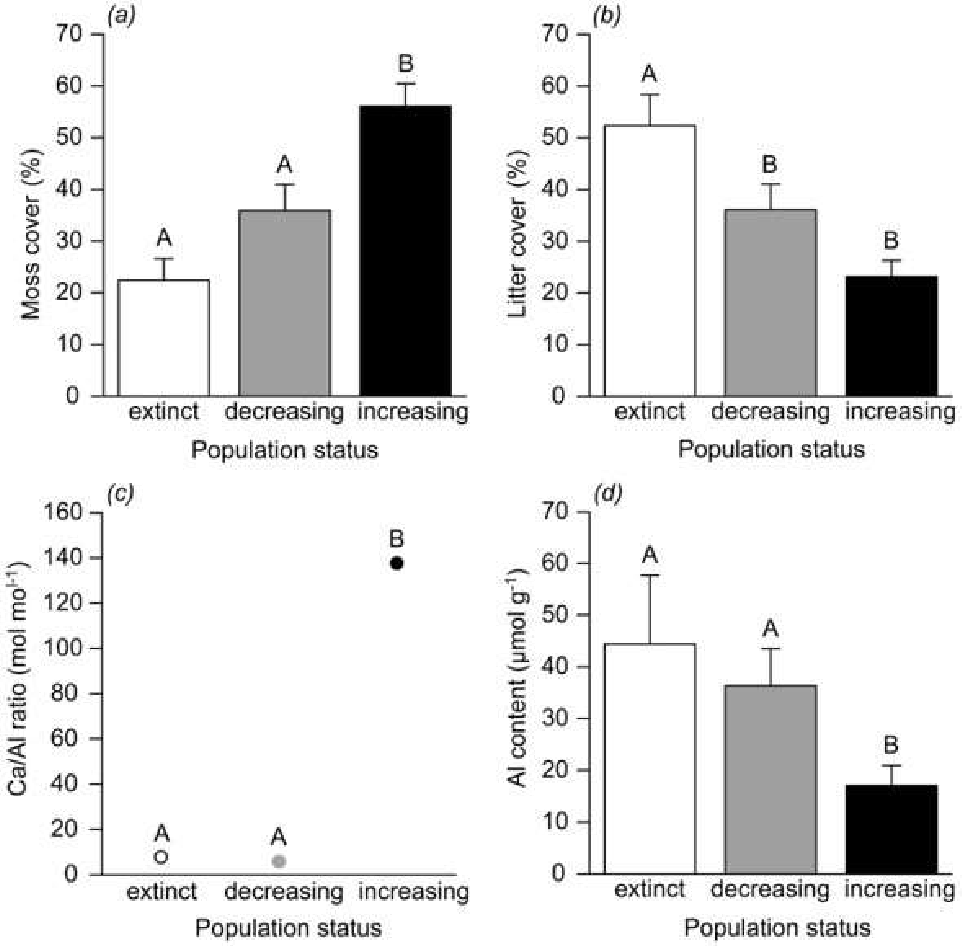

Figure 5. Moss cover *(a)*, litter cover *(b)*, ratio of exchangeable calcium to aluminium in the topsoil *(c),* and Al^{3+} concentration in the topsoil at sites with increasing, decreasing or extinct populations of *P. braunii*. Means and standard errors of each group of three to five populations are given except for the calcium:aluminium ratio, for which the median is given (outliers were excluded). Significant differences ($P < 0.05$) between the three categories are marked with different capital letters

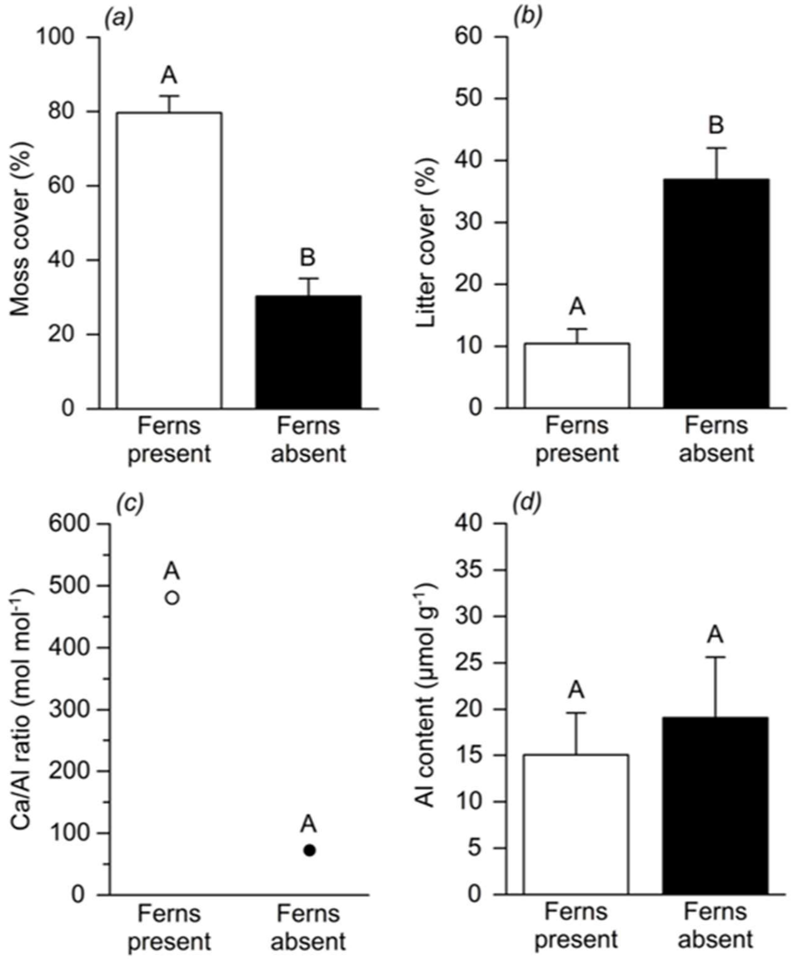

Figure 6. Moss coverage *(a)*, litter coverage *(b)*, ratio of exchangeable calcium to aluminium in the topsoil *(c),* and Al^{3+} concentration in the topsoil at sites with presence or absence of *P. braunii* (increasing populations). Means and standard errors of each group of 32-37 measuring sites are given, except for the calcium:aluminium ratio, for which the median is given. For the latter, outliers were excluded. Significant differences ($P < 0.05$) between the two categories are marked with different capital letters

Table 3.3. Chemical parameters of the topsoil (organic layer Of and Oh horizons and uppermost mineral soil Ah horizon), litter mass, moss layer thickness, soil depth to the bedrock and number of fern species present at locations with increasing, decreasing or extinct populations of *P. braunii* (means and standard error of each group of 3-5 populations).

	Population status		
Parameters	extinct	decreasing	increasing
pH (H_2O)	4.5^{AB} (4.0-6.6)	4.6^{A} (3.7-7.3)	4.8^{B} (3.9-7.8)
N_t (µmol g^{-1})	915.7 (± 143.2)^{A}	467.9 (± 64.2)^{B}	865.3 (± 63.1)^{A}
C_{org} (mmol g^{-1})	17.4 (± 3.0)^{A}	8.9 (± 1.3)^{B}	15.1 (± 1.2)^{A}
C/N ratio	18.5 (± 0.9)^{AB}	18.6 (± 0.4)^{A}	16.9 (± 0.3)^{B}
P_{resin} (µmol g^{-1})	2.8 (± 0.6)^{A}	1.0 (± 0.3)^{B}	2.6 (± 0.3)^{A}
Cation exchange capacity (µmol$_c$ g^{-1})	356.0 (± 58.9)^{A}	216.2 (± 33.5)^{B}	495.3 (± 41.9)^{C}
Base saturation (%)	74.5 (± 7.0)^{A}	71.2 (± 5.1)^{A}	90.7 (± 1.6)^{B}
Litter mass (g m^{-2})	208.4 (± 20.5)^{A}	152.8 (± 27.2)^{B}	89.6 (± 11.1)^{B}
Moss layer thickness (cm)	1.5 (± 0.2)^{A}	3.6 (± 0.5)^{B}	3.7 (± 0.3)^{B}
Soil profile depth to bedrock (cm)	2.8 (± 0.9)^{A}	5.3 (± 0.6)^{B}	3.7 (± 0.4)^{AB}
Number of fern species present	0.7 (± 0.1)^{A}	1.4 (± 0.2)^{B}	1.6 (± 0.2)^{B}

Notes: Different upper-case capital letters indicate significant differences between categories ($P < 0.05$)

Table 3.4. Chemical parameters of the topsoil (organic layer Of and Oh horizons and uppermost mineral soil Ah horizon), litter mass, moss layer thickness, soil depth to the bedrock and number of fern species present at locations with recent occurrence of *P. braunii* (ferns present, increasing or decreasing populations) or nearby sites with absence of the fern (means and standard error of each 42-62 measuring sites).

Parameters	Ferns present	Ferns absent
pH (H_2O)	4.7 (3.9 / 7.8)^A	4.9 (3.9 / 7.4)^A
N_t (µmol g^{-1})	983.3 (± 91.2)^A	740.1 (± 9.3)^A
C_{org} (mmol g^{-1})	16.6 (± 1.7)^A	13.4 (± 1.8)^A
C/N ratio	16.5 (± 0.4)^A	17.2 (0.4)^A
P_{resin} (µmol g^{-1})	2.9 (± 0.3)^A	2.2 (± 0.4)^B
Cation exchange capacity (µmol_c g^{-1})	573.6 (± 71.6)^A	412.1 (± 43.7)^B
Base saturation (%)	91.7 (± 2.3)^A	89.5 (± 2.4)^B
Litter mass (g m^{-2})	56.9 (± 13.1)^A	123.8 (± 16.9)^B
Moss layer thickness (cm)	4.5 (± 0.3)^A	2.9 (± 0.3)^B
Soil profile depth to bedrock (cm)	2.3 (± 0.5)^A	5.3 (± 0.6)^B
Fern diversity (species m^{-2})	2.2 (± 0.2)^A	0.9 (± 0.1)^B

Notes: Different upper-case capital letters indicate significant differences between categories ($P < 0.05$)

Discussion

The role of climatic factors

Both microclimate measurements at sites with increasing, decreasing or extinct fern populations and the analysis of climate trends in the recent past suggest that a sufficiently high air humidity is one of the prerequisites for a thriving *P. braunii* population in Germany. Since all the studied sites are located at montane to upper montane elevation (500-1400 m a.s.l.) in a relatively cool and moist climate with at least 300 mm precipitation in the growing season, our results indicate that soil moisture does not limit fern growth most of the time. This assumption is corroborated by earlier growth chamber experiments conducted with *P. braunii* sporophytes in treatments with contrasting air and soil moisture status (Schwerbrock and Leuschner 2016), which revealed a dominant air humidity and only a small soil moisture effect on the fern's morphogenesis and productivity. Accordingly, *P. braunii* was classified as an obligate air humidity hygrophyte which responded to an increase in the air humidity level from ~65 % to 95 % with a significantly higher leaf conductance, increased aboveground and belowground productivity and higher fertility, even though the soil moisture was kept constant. An important effect of air humidity on the growth of temperate woodland plants that is independent of soil moisture has already been demonstrated in experimental and observational field studies for a number of herbs in broadleaved forests (Leuschner 2002; Lendzion and Leuschner 2009; Leuschner and Lendzion 2009), and for the seedlings of temperate hardwood and coniferous trees (Marsden et al. 1996; Darlington et al. 1997; Lendzion and Leuschner 2008).

That air humidity is an important site factor for the occurrence of *P. braunii* is also suggested by some habitat characteristics in its patchy Central European distribution range. The species is often found at a montane elevation close to watercourses or below dripping water in proximity to or on boulder fields with a northern aspect, where high air humidity and fog are common and exposure to direct sun light is rare. Moreover, in the five regions in the present study the species grows mostly on shallow soil with less than 6 cm or even just 4 cm of fine earth with only very limited soil water reserves. Consequently, the water relations of the ferns should largely depend on the atmospheric moisture status, while rhizomes and roots often have access to only small soil water reserves (Schwerbrock and Leuschner 2017).

Nevertheless, declining and extinct populations in all cases occurred in regions with lower summer rainfall than in regions where increasing populations are found. This suggests that populations are negatively affected by summer rainfall amounts <500 mm, which can act either directly through periodic topsoil desiccation or indirectly via reduced air humidity since lower precipitation and fewer rainfall events are commonly associated with shorter periods of high RH. Decreasing summer rainfall (especially in June) over the last 50 years (data provided by DWD, Offenbach) may have further deteriorated the water status of these populations, probably resulting in reduced survival and/or recruitment and negative population trends.

While no evidence was found that differences in the thermal regime are responsible for the different fate of the *P. braunii* populations, increasing winter temperatures and reduced duration of snow cover may have played a role. As with air humidity, there was a remarkable coincidence between the different population trends and the duration of snow cover at the 13 sites. All populations with a negative trend or recent extinction had snow cover duration <110 days per year. The sporophytes of *P. braunii* have been found to be relatively frost-hardy, tolerating <-20 °C (Sato 1982). However, this does not necessarily mean that the plants are not damaged by repetitive frost exposure as a result of lack of snow cover, as has been found for a number of wintergreen forest understorey ferns (Tessier 2014). The ferns could suffer from frost desiccation in mild winter months, when their overwintering fronds are exposed to an elevated atmospheric demand for water vapour while the soil is still frozen or very cold. In overwintering potted *P. braunii* plants, we observed that frost caused disintegration of the rachis at its base, so that the fronds sink to the ground while remaining attached to the rhizome and staying green. This may increase the chance of being covered by snow and protected from freezing temperatures, and may reduce exposure of the fronds to turbulent air and thus reduce transpirative water losses on frozen soil (cf. Noodén and Wagner 1997). A similar observation has been reported by Schmick (1990), who also found vulnerability of *P. braunii* to late frost events, while a study by de Groot et al. (2012) showed that wintergreen fronds do not necessarily imply the assumed high tolerance of hard winter conditions. The fronds of potted plants in our study experienced pronounced turgor loss during periods of soil frost, but regained turgor in warmer winter periods. Such a response resembles the partly poikilohydric behaviour of some Polypodiaceae species (Kappen 1964; Stuart 1968) or of *Hemionitis palmata* (Kessler and Siorak 2007) and may result in excessive water losses. The assumed importance of snow cover is also reflected in the fact that the sites of extinct populations had the least amount of snow cover (84 days) of all the investigated sites and the location (SA) where ferns disappeared 60 years ago is characterised by a mean snow period of just 50 days. Again, the trend for warmer winters may have deteriorated growing conditions at the sites where snow cover duration is even shorter than it was several decades ago.

The role of moss and litter layers

The results of this study suggest that *P. braunii* benefits from a thicker moss layer and higher moss cover, while the species is less competitive in places with closed leaflitter layers, such as those formed by *Fagus sylvatica* on acid soils. The association of a positive population trend with greater bryophyte cover could be both a direct and indirect effect of the moss layer. Dense moss carpets provide constantly wet growing conditions for the fern's gametophytes and young sporophytes, and may promote spore germination. On surfaces with low stability and shallow soil such as boulders, moss carpets can also provide safe sites for the establishment of both fern generations. A dense moss layer is an indicator of high air humidity (Schmitt et al. 2010) which also favours the growth of woodland ferns such as *P. braunii* (Schwerbrock and Leuschner 2016). Thus, the mosses might only be an indicator of suitable habitat conditions, rather than directly supporting the establishment of the fern.

The apparent negative effect of a closed litter layer on *P. braunii* must be viewed in association with the sensitivity of many temperate forest floor plants to permanent litter cover. While only a few vascular plant species that grow on acid soils are known to benefit from compact and permanently moist litter layers (Ellenberg and Leuschner 2010), many vascular plant species prefer sites with thin or non-permanent litter cover. As a consequence, the diversity of vascular plants and bryophytes typically decreases with increasing cover and depth of the litter layer (e.g. Carson and Peterson 2001; Peintinger and Bergamini 2006; Virtanen and Crawley 2010; Ingerpuu and Sarv 2015). It was assumed that a continuous layer of leaf litter reduces the safe sites needed by *P. braunii* for its establishment. The fact that the litter layer was less extensive in sites with increasing *P. braunii* populations as compared to sites where the species was decreasing or absent would seem to support this conclusion. Thin, rapidly decomposed litter layers are characteristic of the Tilia - Acer ravine forests in which *P. braunii* typically occurs. Beech litter, however, degrades relatively slowly (e.g. Jacob et al. 2010), which are probably less favourable for the fern species. *P. braunii* was only found in beech forests at sites with specific relief and soil conditions where litter could not accumulate, for example on large moss-covered rocks or very steep slopes. The fact that the soil in the studied ravine forests was moderately acidic (pH 4.6 – 4.8) suggests that the decomposition of beech litter is relatively slow and that accumulating litter could in turn promote further soil acidification. This could alter the ravine forest habitats in a way that makes them less suitable for *P. braunii* and other specialised herbaceous plant species. If litter type were indeed an important site factor for this fern species, future changes in tree species composition with forest management could increase the vulnerability of the remaining populations.

Other possibly influential factors

This study's soil chemical data indicate that a higher CEC and higher base saturation at the exchangers in the topsoil had a positive influence on the growth of *P. braunii*, while higher exchangeable Al^{3+} concentrations might be a negative factor. However, it should be noted that CEC and base saturation were relatively high at all sites, as was the ratio of exchangeable Ca^{2+} to Al^{3+}, suggesting that deficiency in basic cations or aluminium toxicity does not play a crucial role for the fern. A more likely explanation of the soil chemistry differences between sites with positive and negative population trends is that all sites with increasing populations are located in ravine forests, in which *Fraxinus, Acer* and other broad-leaved tree species with nutrient-rich and rapidly decomposing litter prevail, while stands with declining *P. braunii* populations are more often found in forests with a significant contribution from *Fagus*, which typically produces less nutrient-rich litter. Litter that is poorer in basic cations and nitrogen is less rapidly decomposed and thus accumulates on the forest floor with an assumed negative effect on fern establishment and/or growth and also on bryophyte cover.

It appears that the fate of *P. braunii* in Germany is closely related to the conservation status of intact *Tilia – Acer* ravine forests. Germany's Red List classifies several such forest communities as 'declining' or 'near-threatened' at a national level, and the situation may be worse in many parts of the low mountain ranges in central and eastern Germany (Schubert et al. 2001). All five increasing populations are located in such more or less intact ravine forests with the presence of a variety of broad-leaved tree species, while three sites with decreasing or extinct populations were found in beech forests on acid soil or other, non-ravine forest communities (Table 3.A1 Appendix). Nevertheless, *P. braunii* seems to be capable of colonising beech forests of the Aceri-Fagetum type at montane and upper montane elevations when the climate is sufficiently moist. It is speculated that these high-elevation beech forests can be colonised when there are boulders or bare rock that are rarely covered by the litter.

Conclusions

This observational study provides relevant information on three environmental factors that may play a key role in the decline and local extinction of the German *P. braunii* populations: decreasing air humidity levels, a reduction in snow cover duration and a shift from a moss-covered to a litter-covered forest floor. Clearly, the striking association of positive population trends with higher air humidity and higher bryophyte cover may be coincidental, possibly masking the effect of other underlying factors. However, the climate chamber experiment of Schwerbrock and Leuschner (2016) provides evidence that air humidity is indeed a critical factor in the survival of the species. The association of decreasing and extinct *P. braunii* populations with forest communities containing abundant beech may also indicate that altered microclimatic conditions or topsoil properties are contributing to the negative population trends of *P. braunii*. Absence of snow cover could enhance the transpirative water loss of the wintergreen fronds during cold periods. It is therefore assumed that the future of *P. braunii* in Germany is probably linked to the existence of habitats that are buffering future macroclimatic changes through their specific microclimate.

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Appendix

Global distribution of P. braunii

P. braunii is an allotetraploid taxon that is apparently of polyphyletic origin with the progenitors being absent. The North American populations show a close relationship to *P. acrostichoides*, a common fern of eastern North America, whereas the European populations seem to be more closely related to an Asian complex (Jorgensen 2012). In Europe, *P. braunii* mainly occurs in the Pyrenees, the Alps, the Carpathians and west Norway, but it is also found in some low mountain regions in Central Europe. The Asian distribution of the fern stretches from central Russia and the Caucasus, across the Altai Mountains and the eastern parts of China and Russia to Japan. In North America, the species is mainly found on the west and east coasts and also in some regions around the Great Lakes (Hultén 1961).

Vegetation analysis

To investigate the sociological affiliation of *P. braunii*, vegetation relevés were conducted in 10 m x 10 m plots containing the target species. All species in the herb, shrub and tree layers were recorded and their cover estimated using the Londo scala. The plant assemblages were assigned to forest communities according to existing forest vegetation surveys (Ellenberg and Leuschner 2010).

Assignment to forest communities

All five locations with increasing fern populations were assigned to communities of the Tilio-Acerion alliance (ravine ash-maple-linden forests, notably the Fraxino-Aceretum pseudoplatani, the Ulmo-Aceretum pseudoplatani and the Aceri-Fagetum associations, which are generally considered to be the fern's characteristic habitats in Germany). However, the *P. braunii* locations at two of the sites with decreasing or extinct populations were found in beech forests (Luzulo-Fagetum) or forest stands without clear assignment to a community type, which may be considered as atypical habitats for this species (Table 3.A1).

Table 3.A1. Assignment of forest stands with presence of *P. braunii* populations with increasing or decreasing status or recent extinction to forest communities (associations).

	Population status		
Community type	extinct	decreasing	increasing
Fraxino-Aceretum Ulmo-Aceretum Aceri-Fagetum	2	3	5
Luzulo-Fagetum Non-assignable stands	1	2	0

Notes: The number of cases is given.

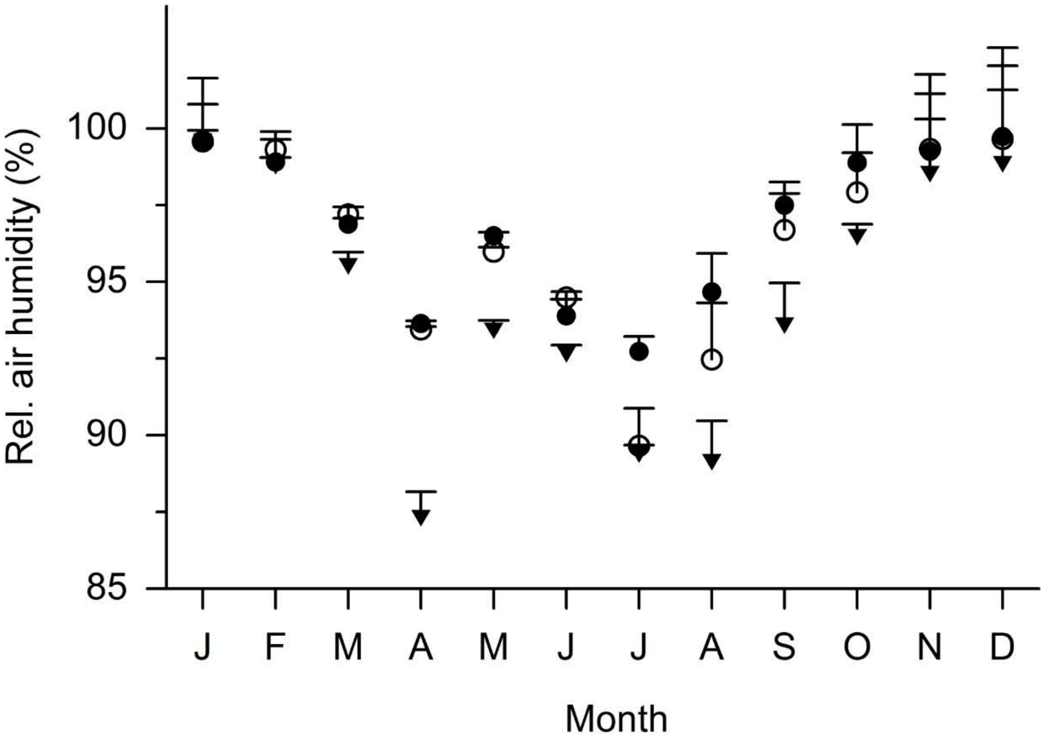

Figure 3.A1. Monthly means of relative air humidity at 80 cm height above ground at locations with increasing (filled circles), decreasing (open circles) and extinct populations (triangles) of *P. braunii* during the period 1 Sept 2012 – 1 Sept 2013. Means of each three to five sites are given with standard error.

CHAPTER 4

Foliar water uptake, a widespread phenomenon in temperate woodland ferns?

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Abstract

Most woodland ferns thrive under conditions of high air humidity, frequent precipitation and exposure to extended periods of leaf wetness, but it is not known how widespread foliar water uptake is in this plant group. In a tracer experiment with deuterated water ($2H_2O$) applied to the leaf surface of five temperate woodland ferns (*Athyrium filix-femina*, *Dryopteris filix-mas*, *Polystichum aculeatum*, *Polystichum braunii* and *Asplenium scolopendrium),* we tested (i) if these species exhibit foliar water uptake and (ii) if the capability to absorb water through the leaf epidermis increases with the frequency of epidermal trichomes. All species had significantly higher abundances of ^2H in tissue water, when extracted distant to the place of application, compared to the background level (0.052 – 0.504 atomic % vs. 0.015 atomic %), evidencing uptake through the epidermis and leaf-internal translocation. A positive relation between trichome density and ^2H incorporation was found only for the second-order pinnae but not for the more central frond sections. The results suggest that foliar water uptake may be widespread among temperate woodland ferns across different families and that leaf trichome structure probably is influencing this process.

Key words: Asplenium, Athyrium, deuterated water, Dryopteris, Polystichum, trichomes

Introduction

Various studies have shown that foliar water uptake can play an important role for the water relations of terrestrial plants (e.g. Stone 1950; Grammatikopoulos and Manetas 1994; Boucher et al. 1995; Breshears et al. 2008; Limm and Dawson 2010; Mayr et al. 2014). The ability of plants to absorb liquid water through leaves turned out to be particularly widespread in moist environments and in ecosystems, where besides rainfall, additional water is supplied by mist, fog and dew that cause frequent leaf wetting (Limm et al. 2009; Goldsmith et al. 2013). Yet, our knowledge of the process of foliar water uptake is still rudimentary.

Ferns and fern-allies reach their highest species diversity in environments with high air humidity (Kessler et al. 2011), and the growth, vigor and morphogenesis of woodland ferns occurring in humid habitats seem to be closely related to the atmospheric water status (Schwerbrock and Leuschner 2016). The dependence on moist air seems to be particularly high in poikilohydric ferns such as filmy ferns (Hymenophyllaceae; Proctor 2012) but was also proven experimentally for members of the Dryopteridaceae (Schwerbrock and Leuschner 2016). While many temperate ferns thrive in shady and moist woodlands, where air humidity is generally high, other ferns grow on sun-exposed shallow soils with generally low water availability. Both ecological groups could have in common that the atmospheric water status likely plays an important role for the ecology of these species. A large influence of atmospheric water was demonstrated for several shallow-rooted desert angiosperms that are largely dependent on the amount of dew (Hill et al. 2015). Close association with the atmospheric water sources might also exist in plants growing in humid climates, if water availability is limited by a low soil moisture-storing capacity of shallow soils (Moles et. al 2014). For example, the temperate woodland ferns *Polystichum braunii* (Spenn.) Fée), *Cryptogramma crispa* (L.) R. Br. Ex Hook. or *Woodsia ilvensis* (L.) R. Br. often occur in Central Europe on shallow soils over boulders, suggesting that these species should face water shortage if atmospheric water is not used. However, not much is known about foliar water uptake by ferns (Pessin 1924; Stuart 1968; Matthes-Sears et al. 1993; Limm et al. 2009). Yet, there are reports for some species about the absorption of dripping fog or dew via their fronds, which seems to help withstanding drought periods (e.g. Stone et al; 1950, Eller et al. 2015;

Munné-Bosch 2010; Fu et al. 2015). This indicates a certain role of foliar water uptake in ferns, but systematic studies using experiments are still lacking.

The morphological and chemical properties of the cuticle influence the wettability of the leaf surface (Koch and Barthlott 2009) and they should also determine how much water can be absorbed by a leaf. The cuticle’s permeability for water has been found to vary with the ecology of the plant species and their occurrence in different climates (Schreiber and Riederer 1996; Limm and Dawson 2010). However, trichomes could also influence how much water is penetrating through the leaf surface. Certain trichomes appear to retain the water on the leaf surface, thereby likely reducing evapotranspiration rate and/or wettability (Grammatikopoulos and Manetas 1994; Munné-Bosch 2010; Fernández et al. 2014). The fact that poikilohydric ferns often exhibit high trichome densities (Kessler and Siorak 2007) suggests that these laminar structures play a certain role in the water status of the fronds (e.g. Pessin 1924; Stuart 1968; Müller et al. 1981; Tsutsumi and Kato 2008). Indeed, for some species, water uptake through trichomes is likely (Metthes-Sears et al. 1993). In some ferns, it was found that the density of trichomes increases with air humidity (e.g. Halloy and Mark 1996; Kluge and Kessler 2007; Schwerbrock and Leuschner 2016) and thus with the duration of leaf wetness. Nevertheless, the functions of trichomes in ferns, which show considerable morphological variability among species, are still mostly unclear (Halloy and Mark 1996; Watkins 2006; Kluge and Kessler 2007).

In this study, we used deuterated water as a tracer to explore, whether the sporophytes of five terrestrial Central European woodland ferns from three different families (Aspleniaceae: *Asplenium scolopendrium* (L.); Dryopteridaceae: *Dryopteris filix-mas* (L.) Schott, *Polystichum aculeatum* (L.) Roth, *Polystichum braunii*; Woodsiaceae: *Athyrium filix-femina* (L.) Roth) feature foliar water uptake. According to the findings of earlier studies, we tested (i) if these species exhibit foliar water uptake and (ii) if the capability to absorb water via the fronds increases with the frequency of adaxial leaf trichomes.

Materials and methods

Plant material and experimental protocol

The ferns used in the experiment were obtained from the Botanical Gardens of the Universities of Göttingen and Kassel (Germany) which had reared the plants from spores collected in different forest regions of Germany. *A. filix-femina* and *D. filix-mas* are common in Central Europe from the lowlands to the alpine belt, thriving in the understory of woodlands that provide cool, moist and shady conditions. *P. aculeatum* prefers similar habitats but is distributed more patchily. This is also the case in *A. scolopendrium* which often grows on moist rock faces located in shady forests. *P. braunii* is one of the rarest ferns of Central Europe with only a few occurrences. This fern with dependence on high air humidity (Schwerbrock & Leuschner 2016) is restricted to deeply shaded, steep and rocky ravine forests of the lower montane to upper montane belt in the uplands and the Alps. Six mature fertile plants per species of similar size (maximum height ca. 50 cm) were used in the experiment. They were containerized individually in large 10 L-pots (diameter = 29 cm) filled with a peat-sand mixture (70/30 v/v) to provide sufficient soil volume for avoiding root space limitation and placed in a glasshouse for acclimatization four weeks before the start of tracer application. During the experiment, the soil was irrigated daily to keep moisture high and avoid periods of water shortage. The climatic conditions in the greenhouse were kept constant at about 20 °C and 80 % relative air humidity during the day. Shade cloth with a light transmissivity of 40 % was installed above the plants to provide a homogeneous light regime and protect the plants from excess radiation.

The tracer experiment with application of heavy water ($2H_2O$) to the foliage of sporophytes of *A. filix-femina*, *A. scolopendrium*, *D. filix-mas*, *P. aculeatum* and *P. braunii* was conducted in the Experimental Botanical Garden of the University of Göttingen in May/June 2014. We used $2H_2O$ with 99.95 atomic % ^2H (MagniSolv, Merck, Darmstadt, Germany), which is an about 7000-fold higher deuterium concentration than in atmospheric water (0.015 atomic %, IAEA 2001). A thin water film was applied to different frond sections to simulate leaf wetting. Subsequently, adjacent leaf tissue was cut out and the water extracted with cryogenic vacuum distillation; the water was then analyzed for its $^1H/^2H$ ratio by isotope ratio spectroscopy.

The deuterated water was applied to the epidermal surface of three different frond sections; tissue directly near the primary rachis, near the secondary rachis and of the second-order pinnae of fully developed upper fronds of the four *Athyrium, Dryopteris* and *Polystichum* species with pinnate leaves. In case of the fronds of *A. scolopendrium* with entire margins, we applied tracer only to the primary rachis in the section of the fronds. Each treatment was replicated with six different individuals per species. In the experiments with the primary rachis, the water was applied with a 100 µL Eppendorf pipette (Sigmar-Aldrich, Munich, Germany) by dripping ca. 70 µl $2H_2O$ solution evenly on a surface section of ca. 20 cm length between the first basal leaflet and the frond apex. We subsequently harvested the leaflets inserting in that section of the primary rachis, where the tracer had been applied (Fig. 1A). In the experiments with the secondary rachis, a 10 µL syringe (800 Series, Hamilton, Bonaduz AG, Switzerland) was used to apply 100 µL of $2H_2O$ to several leaflets per sample and the distal sections of the second order pinnae were subsequently harvested (Fig. 1B). Finally, about 400 second-order pinnae were treated with 400 µL $2H_2O$ per sample using a 1-10 µL Eppendorf Multi-pipette (Sigmar-Aldrich, Munich, Germany) applying a single drop on each pinnae. In these experiments, only the apex of the sub-leaflets (about a third of the pinna area) was harvested (Fig. 1C). The variable amount of tracer applied to the three types of leaf sections results from the different size of the target areas and the contrasting amounts of leaf tissue harvested for the isotope analysis. The deuterated water remained for 30 min on the leaf surface before leaf samples were harvested. We estimate that the water film was about 0.5 mm thick resembling the amount of intercepted water which remains on fern fronds after a saturating rain event. We assumed that if intercepted water diffuses through the cuticle into the leaf tissue, this should happen primarily within the first 30 min; otherwise, evaporation of intercepted water rapidly reduces the thickness of the water film when the evaporative demand is high.

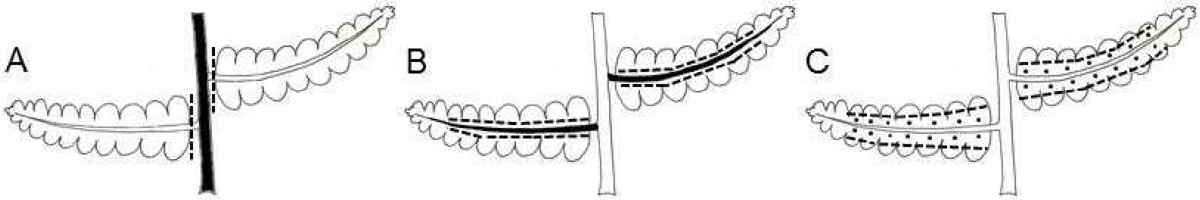


Figure 4.1. Schematic illustration of the three types of frond sections to which the 2H2O tracer was applied (black areas) and the leaf tissue that was cut for isotopic analysis 30 min after application (frond sections distal to the dotted lines). (A) primary rachis (A), secondary rachis (B), and second order pinnae (C)

The leaf tissue used for isotopic analysis was in all cases cut out of the frond with a scalpel at a location about 0.5-2 cm distant to the spot of tracer application in distal direction (Fig. 1). By this approach, any contamination of the sample with tracer solution was avoided and significant transport of infiltrating water in the leaf tissue must have happened before harvest. In all cases, 2 g of fresh tissue material was cut out, transferred into glass vessels and processed for 30 min at 80 °C and at a suction of $10^0 – 10^{-1}$ hPa (following West et al. 2006) through a cryogenic vacuum distillation chain with a custom-made system after Orlowski et al. (2013). The leaf tissue extracts were filled into 0.5 ml glass vials and stored at 4°C until sent to the Center for Stable Isotope Research at the University of Göttingen, where the ^{1}H and ^{2}H concentrations were determined by isotope ratio mass spectrometer (IRMS) using a *MAT 253 mass spectrometer combined with a Conflo IV Interface (TC-EA) from Thermo-Fisher Scientific (Bremen).* The background ^{2}H concentration in leaf tissue was analyzed in six individuals per species in similar frond sections as studied in the experiment (control). In addition, the ^{2}H abundance in the tap water used for irrigation was determined (n = 6). Possible differences in the isotopic signature between different plant parts as reported by Wang et al. (1995) should be of minor importance in our study, since all samples processed were taken from the sub-leaflets (except for *A. scolopendrium* with leaves with entire margins). To exclude the influence of diurnal variation in stomatal aperture and different transpiration rates on the ^{1}H/^{2}H signature of the leaves (Gat 2010), we conducted all experiments at the same daytime between 09:00 and 12:00 a.m. in the glasshouse with constant microclimate.

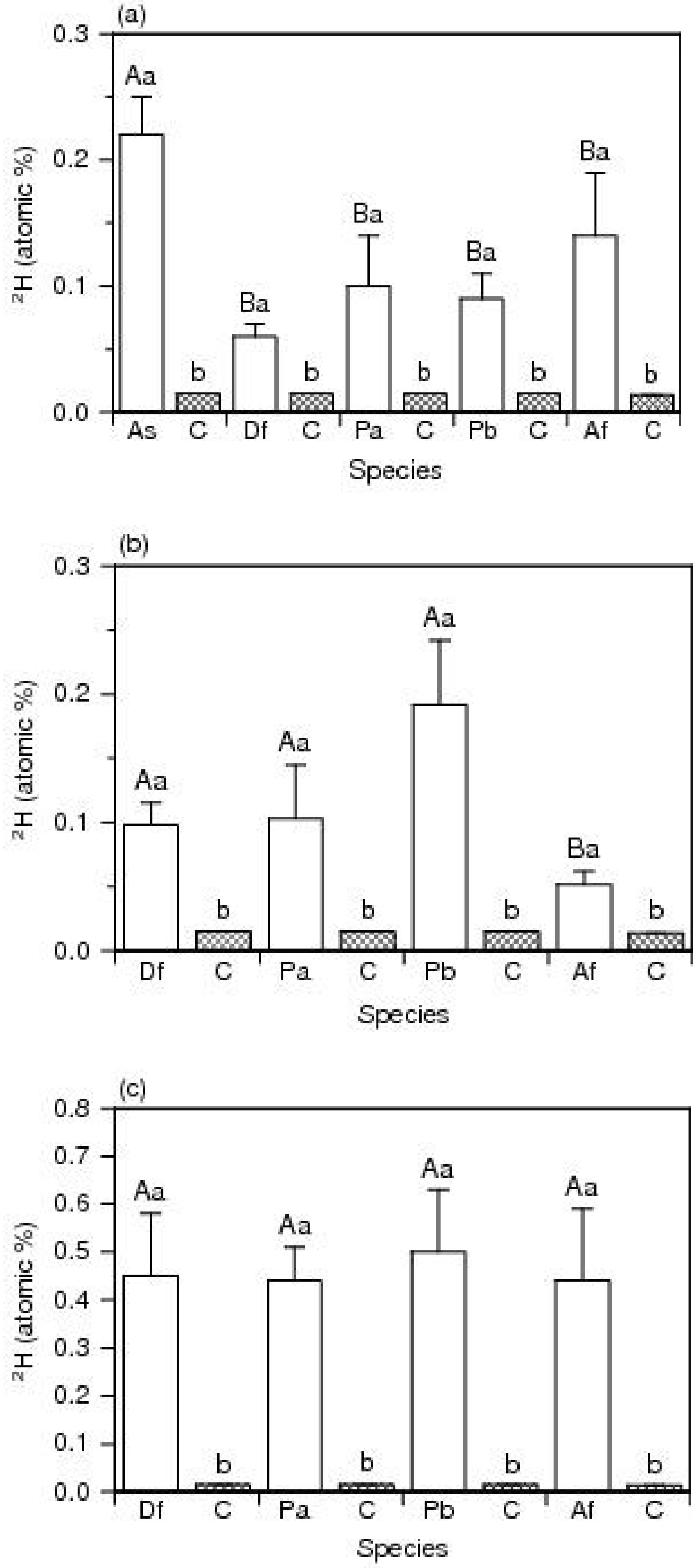
The frequency of trichomes was determined for all three investigated frond sections (primary rachis, secondary rachis and second-order pinnae) by counting them with a dissecting microscope within seven ca. 5.0 mm^2–large squares (ca. 2.2 mm x 2.2 mm) on the primary rachis and the second-order pinnae, and within seven ca. 2.5 mm^2–large squares (ca. 1.6 mm x 1,6 mm) on the secondary rachis in each seven individuals per species. The figures were extrapolated to 1 cm^2.

Statistical analyses

Statistical analyses were performed with the software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). All data were tested for normal distribution by the Shapiro-Wilk test. Due to non-normal distributed data and inhomogeneity of variance (Levenscher test) a Kruskal-Wallis test was applied to test for significant differences in the deuterium content between the three treated frond section types and the untreated control, and between corresponding frond sections of the different species. No statistical test was applied to differences in the isotope signature of the three different frond sections of a species due to variable tracer amounts applied to the different target areas and variable distances between the site of application and the tissue section collected for isotope analysis. Regression analyses were carried out with the software Xact 7 (SciLab, Saint-Yrieix, France).

Results

All 13 treatments with $2H_2O$ application (3 frond section types, 5 species) showed a markedly higher ^2H abundance in the leaf tissue after tracer application to the epidermis than the untreated control (Fig. 4.2a, b, c; all differences to control significant at $P < 0.01$). The mean values ranged from 0.052 (± 0.010) to 0.504 (± 0.128) atomic % ^2H, while the mean of the controls across the five species was 0.015 % (±0.000), matching the measured concentration in the tap water used for irrigating the plants and the natural abundance of ^2H in the atmosphere. As an average across the four species with pinnate fronds, the second-order pinnae had about 30 times higher ^2H abundances than the control, the tissue near the second-order rachis 7.5 times, and the tissue near the first-order rachis 6 times higher deuterium abundances than the control. In *A. scolopendrium*, for which only tissue close to the first-order rachis was investigated, the enrichment compared to the control (about 15 times higher) was significantly larger in this tissue than in first-order rachis tissue of the other species with pinnate leaves ($P < 0.05$; Fig. 4.2a). The highest tracer incorporation was found for the second-order pinnae. In this frond section, no significant difference in ^{2}H incorporation existed between the species (Fig. 4.2c). With respect to the secondary rachis, *A. filix-femina* had a significantly smaller incorporation than the other four species (Fig. 4.2b).


(a)
0.3
0.2
0.1
0.0
²H (atomic %)
Aa
b
Ba
b
Ba
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Ba
b
Ba
b
As
C
Df
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Pa
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Pb
C
Af
C
Species
(b)
0.3
0.2
0.1
0.0
²H (atomic %)
Aa
b
Aa
b
Aa
b
Ba
b
Df
C
Pa
C
Pb
C
Af
C
Species
(c)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
²H (atomic %)
Aa
b
Aa
b
Aa
b
Aa
b
Df
C
Pa
C
Pb
C
Af
C
Species

Figure 4.2. Abundance of ^2H (in atomic %) in the water extracted from the leaf tissue after application of ^2H_2O on *(a)* the primary rachis, *(b)* secondary rachis and *(c)* second-order pinnae of *Asplenium scolopendrium*, *Dryopteris filix-mas*, *Polystichum aculeatum, Polystichum braunii* and *Athyrium filix-femina* (means ± SE, n = 6). Different small latters indicate significant differences between treatment and control ($P < 0.01$). Different capital letters indicate significant differences between the species for a given organ ($P < 0.05$). The control was not treated with $2H_2O$; the value given is the natural abundance of ^2H in the frond tissue of the species

The trichome density on the primary rachis, the secondary rachis, and the second-order pinnae varied between 145 cm^{-2} (*A. filix-femina*) and 900 cm^{-2} (*P. braunii*), 165 cm^{-2} (*D. filix-mas*) and 715 cm^{-2} (*P. braunii*), and 5 cm^{-2} (*A. filix-femina*) and 175 cm^{-2} (*P. braunii*), respectively. A positive relation between the amount of tracer incorporated into leaf biomass and the density of trichomes on the upper frond surface was found for the experiment with second-order pinnae ($P < 0.001$; Fig. 4.3c) and, at marginal significance, for the tissue close to the second-order rachis ($P = 0.052$; Fig. 4.3b), but not for the tissue adjacent to the first-order rachis (Fig.4.3a).

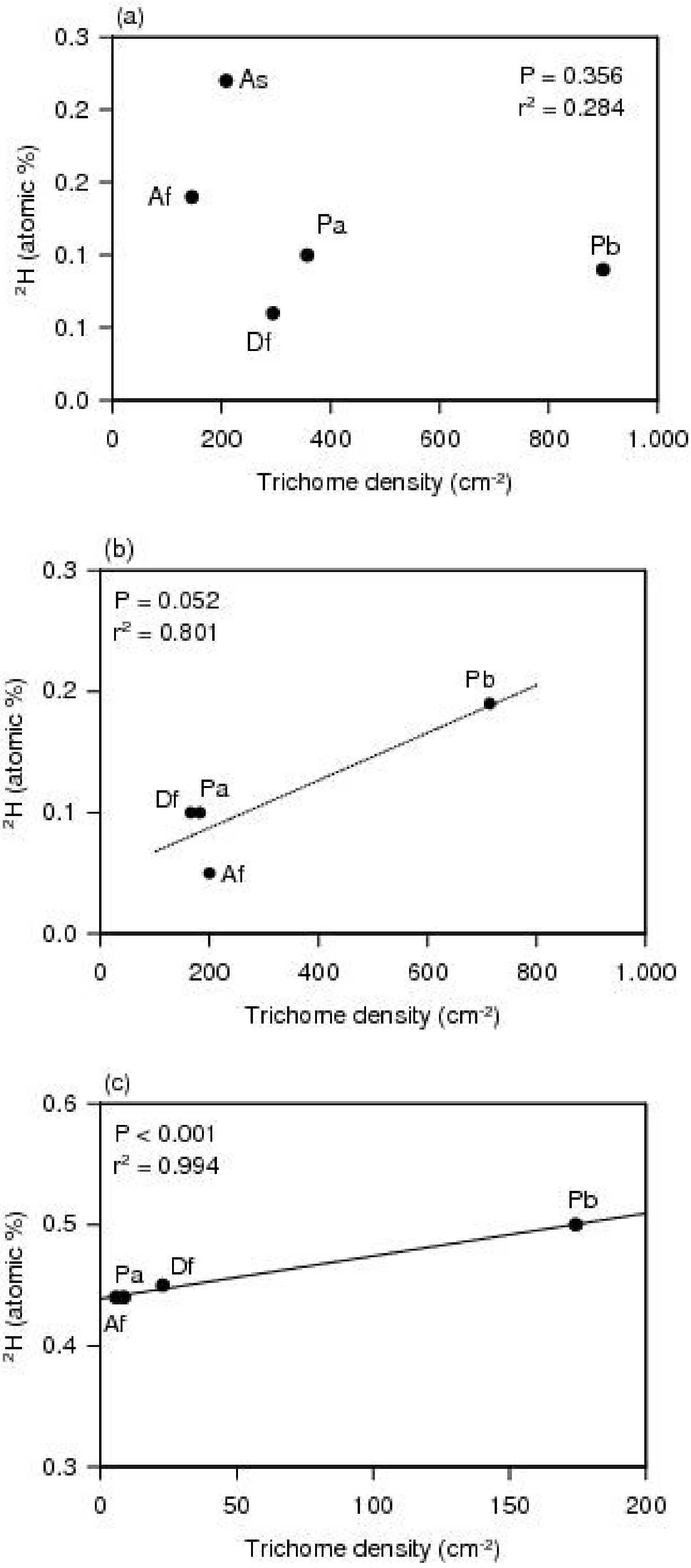
(a)
0.3
0.2
0.2
0.1
0.1
0.0
As
P = 0.356
r^2 = 0.284
Af
Pa
Pb
Df
0
200
400
600
800
1.000
Trichome density (cm^{-2})
^{2}H (atomic %)
(b)
0.3
0.2
0.1
0.0
P = 0.052
r^2 = 0.801
Pb
Df
Pa
Af
0
200
400
600
800
1.000
Trichome density (cm^{-2})
^{2}H (atomic %)
(c)
0.6
0.5
0.4
0.3
P < 0.001
r^2 = 0.994
Pb
Pa
Df
Af
0
50
100
150
200
Trichome density (cm^{-2})
^{2}H (atomic %)

Figure 4.3. Dependence of the ^2H abundance (in atomic %) in the leaf tissue 30 min after the application of $2H_2O$ on *(a)* the primary rachis, *(b)* the secondary rachis and *(c)* the second-order pinnae on the density of trichomes (n cm^{-2}) on the treated frond epidermis (means ± SE, n = 6). *As = Asplenium scolopendrium*, *Df = Dryopteris filix-mas*, *Pa = Polystichum aculeatum, Pb = Polystichum braunii, Af = Athyrium filix-femina, C = control*. Significant (continuous lines) and marginally significant trend lines (dotted) are depicted

Discussion

Our experiments convincingly demonstrate the uptake of liquid water through the epidermis of sporophyte fronds of five temperate woodland fern species. Two of the species (*A. filix-femina*, *D. filix-mas*) are common in European broad-leaf and coniferous forest communities, while the other three occur more locally or are rare (*P. braunii*). Our sampling protocol with a distance between the location of tracer application on the frond and tissue collection allows excluding contamination as a cause of ^2H enrichment in the leaf tissue. The tracer must have diffused through the cuticle and epidermis and subsequently was transported in the leaf over distances of ≥0.5 cm to the collected tissue at more distal position. This was the case in all 78 samples treated with ^2H_2O, which consistently showed significantly higher deuterium concentrations in the tissue water than in that from the control plants with deuterium background levels. As sampling took place 30 min after tracer application, uptake and leaf-internal transport must occur rapidly and thus this is a relevant process even after rain showers with only relatively short duration of surface wetness.

Even though our results are only a qualitative proof of foliar water uptake in ferns, but do not give information on the amount of water absorbed, this evidence is valuable, as it points at the possibly widespread utilization of this additional water source by woodland ferns. Foliar uptake of liquid water has been proven for other fern species (Limm et al. 2009; Goldsmith et al. 2013; Metthes-Sears et al. 1993) and a number of angiosperms (Stone and Went 1957; Gouvra and Grammatikopoulos 2003; Zimmermann et al. 2007; Cassana and Dillenburg 2012; Eller et al. 2013; Cassana et al. 2016), but was not investigated for a larger number of common or rare woodland ferns with variable frond morphology and growth habit. From the fact that foliar water uptake has already been proven for a variety of plant species of contrasting phylogenetic position (lichens, mosses, ferns, gymnosperms, herbaceous and woody angiosperms) and for different environments (perhumid cloud forests, temperate and Mediterranean forests, semi-arid to arid ecosystems), our results are not surprising, but this phenomenon has not received much attention in fern ecology in the past. Our results help to achieve a more complete picture of the importance of foliar water uptake in different vegetation types (Limm et al. 2009).

Ferns may profit from foliar water uptake in environments with contrasting hydrologic regimes. In mesic lowland to montane forests, intercepted rainfall (and snow) could be taken up in periods of leaf wetness, until evaporation has drained the leaf surface. How much water is incorporated depends largely on the frequency of rainfall events and the evaporative demand in the period following a rain shower. In cloud forests of upper montane elevation, intercepted fog could be another important foliar water source. Finally, dewfall (mostly during nighttime hours) and spray water close to waterfalls may represent other water sources available for foliar uptake. How important these sources are, is largely determined by the duration of leaf wetness on the fronds.

Comparing the five species in our study suggests that trichome density on the epidermis may play a role in water uptake, but apparently only in the most distal frond sections, i.e. the secondary pinnae. The correlation found between trichome density and ^{2}H abundance in leaf tissue cannot be taken as a proof that these structures are indeed involved in foliar water uptake, as different species with different trichome types were compared and the isotope data do not give uptake rates. Moreover, the species certainly differ with respect to leaf morphology and possibly also foliar water content. Observed species differences in the abundance of absorbed ^{2}H (as visible in *A. scolopendrium,* Fig. 2a) can thus not be interpreted in terms of differential use of foliar water. Yet, several authors have speculated about a role of trichomes in foliar water uptake in both angiosperm and fern species (Munné-Bosch 2010; Grammatikopoulos and Manetas 1994; Eller et al. 2013; Fernandez et al. 2014). In our study, the rare woodland fern *P. braunii* with very high trichome density also exhibited the greatest ^{2}H abundance after tracer application. Moreover, sporophytes of this species produced significantly more epidermal trichomes when grown in climate chambers with high relative air humidity (95 %) compared to lower humidity (65 %; Schwerbrock and Leuschner 2016). Without doubt, the significant relationship between ^{2}H incorporation and trichome density on the second-order pinnae is largely dependent on the specific behavior of *P. braunii* in the experiments. This relationship thus requires further testing in additional experiments with more fern species. Nevertheless, the lacking or only marginally significant relationship between trichome density and ^{2}H incorporation on the primary and secondary rachis as compared to the second-order pinnae, and additional information on trichome function published earlier, may point at different functions of these organs (Kluge et al. 2007). While the typically scale-like trichomes on the primary rachis probably mainly have protective functions (Müller et al. 1981), e.g. against excess radiation (Watkins 2006), high transpiration or cold in winter, and thus rather should prevent surface wetting, the trichomes on the adaxial side of the second-order pinnae are filamentous. They could either be active in water conduction toward the epidermis, serve for water storage (Tsutsumi et al. 2008), or help to retain water on the epidermal surface, thus extending the period in which diffusion through the cuticle can take place. Riederer and Schreiber (2001) found that the amount of water transported across plant cuticles does linearly increase with time.

Our study demonstrates that the morphology of the fern fronds may also influences foliar water uptake. Another possible factor influencing foliar water uptake capacity apart from trichome density and shape is variation in the structure and chemical properties of the cuticle causing differences in the permeability of different frond types, but also of different frond sections. The more distal pinnae could perhaps have higher transpirative water losses with greater diurnal leaf water potential drops than sections close to the rachis, which could promote water diffusion across the epidermis along enhanced water potential differences (Rundel 1982). Finally, one may speculate that differences in leaf tissue water potential across the frond contribute to gradients in uptake (Limm et al. 2009). Uptake experiments together with water potential measurements have to show whether tissue water status is a factor influencing water uptake from the leaf surface of ferns.

Conclusion

In confirmation of our first hypothesis, this study provides evidence that the sporophytes of all five fern species studied are capable of rapid liquid water uptake through the frond surface. This suggests that foliar water uptake may be widespread among temperate woodland ferns across different families. We obtained some evidence that trichomes may play a role in foliar water uptake, partially supporting our second hypothesis, but detailed physiological studies are needed and more species have to be investigated to confirm this assumption. Further experimental studies should compare ferns of different ecosystem types and climatic conditions and should address the influence of the type of water source (rainfall, dew, fog, mist, splash water) on uptake, as was done, for example, by Hill et al. (2015). By comparing ferns cultivated with and without leaf wetness, growth and leaf water status could be studied in their dependence on foliar water uptake.

A deeper understanding of the functional role of foliar water uptake in ferns and other plant groups is an important step to increase the predictive power of models simulating the biological consequences of climate warming-related droughts. If a significant proportion of the water consumed by plants is contributed by foliar uptake, changes in rainfall duration and frequency and air humidity (evaporative demand) may be biologically more relevant than altered precipitation amounts.

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CHAPTER 5

Synthesis

Synthesis

The observational study in the four remaining regions with occurrence of *P. braunii* in Germany where climatic, edaphic and phytosociological characteristics of sites with increasing, decreasing or recently extinct fern populations were compared, provided hints to the assumption that two factors may play a key role as drivers of population decline and local extinction of the species: decreasing air humidity levels and a shift from a moss to a litter-covered forest floor (chapter 3). Before a more detailed explanation of these phenomena, some of the results of the tracer study with heavy water (chapter 4) and the three-factorial climate chamber experiment (chapter 2) need to be discussed. Both studies provided important key information about the ecology of *P. braunii* and played a decisive role in the analysis of potential threats for the species.

The climate chamber experiment showed that nine of 11 morphological, physiological and growth-related traits of *P. braunii* were significantly influenced by an increase in RH from 65% to 95%, leading to higher leaf conductance, increased above- and belowground productivity, higher fertility, more epidermal trichomes and fewer leaf deformities under high air humidity. In contrast, soil moisture variation (from 66% to 70% in the moist to ca. 42% in the dry treatment) influenced only one trait (specific leaf area), and temperature variation (15 °C versus 19 °C during daytime) only three traits (leaf conductance, root/shoot ratio, specific leaf area); RH was the only factor affecting the productivity. Contrary to our expectations, an increase in summer temperatures, as simulated by the 4 °K temperature elevation, a value frequently predicted by region climate scenarios for the end of the 21st century (IPCC 2013), does not seem to affect the vigor of the fern. The apparently high dependence of *P. braunii* on low VPD levels can be viewed in association with some general physiological and morphological characteristics of pteridophytes that may render ferns more susceptible to dehydration caused by reduced atmospheric moisture. Fern stomata in apparent contrast to angiosperms, for example, seem to act rather as passive hydraulic valves, closing when leaf and guard cells dehydrate (Brodribb and McAdam 2011), while active stomatal closure, despite having been proven for pteridophytes (Doi et al. 2006, Doi and Shimazaki 2008), seems to be less pronounced in this group. Moreover, fern leaf growth is caused by apical meristematic cells instead of leaf basal meristems located on the tips of fronds, relatively distant from the soil water reservoir and linked to it through a relatively long flow path via roots, rhizomes and the base of the frond. A possible mechanism of a dry air effect on leaf morphogenesis and growth is VPD (vapor pressure deficit) control of foliar water loss, which should affect cell turgor in apical meristems, the leaf epidermis and in other leaf tissues, and resulting in hydro-passive reduction in stomatal conductance, with a negative effect on CO_2 assimilation. Field measurements of Benemann (1996) confirm that *P. braunii* plants under natural conditions already maintain relatively low leaf conductances. *P. braunii* furthermore thrive in an atmosphere with vapor saturation or high RH while plants originating from such climates tend to reduce the synthesis of cuticular waxes resulting in an even closer coupling of the epidermal water status to the atmosphere. The end of this development is represented by poikilohydric ferns, such as some Hymenophyllidaceae. They only possess a very thin cuticle or none at all which allows efficient water and nutrient uptake over the leaf surface but high dependence on the atmospheric moisture status (Proctor 2012). The high dependence on high air humidity of *P. braunii* may also be explained by the habitat types of the species in Central Europe. Populations are often found close to water streams or waterfalls in foggy areas where RH might partly compensate for shallow soils. This matches the results of the tracer experiment with deuterated water (chapter 4) in which foliar water uptake was proved for *P. braunii* and for other temperate woodland ferns (*Athyrium filix-femina*, *Dryopteris filix-mas*, *P. aculeatum* and *A. scolopendrium*). All species showed a significantly higher abundance of ^{2}H in tissue water, when extracted distant to the place of the tracer application (^2H_2O), compared to the background level (0.052 – 0.504 vs. 0.015 atomic % ^{2}H), evidencing uptake of liquid water through the epidermis by different frond organs (second-order pinnae, primary rachis and secondary rachis) and leaf-internal translocation. The positive relation between trichome density and ^{2}H incorporation for the second-order pinnae together with the fact that *P. braunii* with the highest trichome density on this frond sections also exhibited the greatest ^{2}H abundance (see Fig.2-3 in chapter 4) suggest that these laminar indumenta types may play an important role for the water relations of the fern. The trichomes could either be active in water conduction toward the epidermis, or help to retain water on the epidermal surface, thus extending the period in which diffusion through the cuticle can take place (cf. Riederer and Schreiber 2001). We conclude that foliar water uptake may be widespread among temperate woodland ferns across different families, but it is largely dependent on the epidermal surface structure and the functionality of the frond section. The enhanced capability of *P. braunii* for foliar uptake rather supports the assumption that high atmospheric moisture might compensate for the shallow soils in rock outcrops with only little fine fraction and low water storage capacity (cf. Bennert 1999).

The relationship between decreasing air humidity levels and the fading of P. braunii in Germany

The close relationship of the species to low VPD levels proved by the results of the climate chamber experiment, together with the findings of the tracer study with heavy water which suggest that the species' characteristic filamentous leaf trichomes (see Fig. 1a in chapter 1) may enhance foliar water uptake, leave no doubt that the atmospheric water regime represents a crucial factor for *P. braunii*. In fact, the observational study showed that the mean growing season VPD tended to be larger, and the frequency of dry-air episodes (relative air humidity <60%) significantly higher in sites with decreasing or extinct populations than in stands with increasing population size. Shorter periods of high RH are commonly the result of a lower precipitation and sites with decreasing or extinct populations were characterized by less summer rainfall (<500 mm from May-Sept) than is the case with sites with increasing populations (generally >550 mm). Precipitation in May and June, when the ferns are sprouting, was below 100 and 125 mm, respectively, in the populations with a negative trend (decreasing and extinct) but ranged between 100 and 200 mm in May and 140 and 250 mm in June at the locations with increasing populations. The most critical influences of low RH are probably represented by the disturbance of the leaf budding and/or the suppression of the complete unroll of the second-order pinnae in the early growth period of the plants (May and June). Both effects also cause a reduction of the sori bearing frond sections resulting in a decrease of the fertility. This was shown by the climate chamber experiment with significantly increasing leaf deformities and a decreased fertility when air humidity was reduced from 95% to 65% ($P < 0.01$, see chapter 2 Fig. 2.1b-d). Fewer rainfalls, fog, mist and dew events, moreover decreases in the duration and the frequency of leaf wetting events which, in turn, reduce the amount of water absorbed by the leaves. It is possible that populations are negatively affected by growing seasons precipitation <500 mm and that decreasing summer rainfall due to climate change during the last 50 years, especially in May and June when the ferns sprout, may have deteriorated the water status of these populations, likely resulting in reduced survival and/or recruitment and in negative population trends. The fact that the Saxon Uplands (Eastern Germany) with several extinct populations and no recent occurrence of the fern, featured the lowest precipitation amounts (<400 mm) of all regions supports this conclusion.

The effect of a shift from a moss to a litter-covered forest floor on P. braunii

The observational study showed that positively developing populations of *P. braunii* were characterized by significantly higher moss coverage (56% of the forest floor) but smaller cover by tree litter layer (23%) compared to decreasing (36% and 38%) or recently extinct populations (22% and 52%). An observational study by Kessler (2001) suggests that bryophyte cover on the forest floor may be an important factor which strongly correlates with fern species richness. The association of a positive population trend with a higher bryophyte cover, however, could be either a direct or an indirect effect of the moss layer. Dense moss carpets provide constantly wet growing conditions for the fern's gametophytes and young sporophytes and they may promote spore germination. On surfaces with low stability and shallow soil, such as boulders, moss carpets can also provide safe sites for the establishment of both fern generations. On the other hand, a dense moss layer is an indicator of high air humidity (Schmitt et al. 2010) which is also favors the growth of woodland ferns such as *P. braunii*. The apparent, negative effect of a closed litter layer on *P. braunii* must be discussed under the aspect of the sensitivity of many temperate forest floor plants to permanent litter coverage. The diversity of vascular and bryophyte plants typically decreases with increasing coverage and depth of the litter layer (e.g. Carson and Peterson 2001, Peintinger and Bergamini 2006, Virtanen and Crawlay 2010, Ingerpuu and Sarv 2015). However, the fact that the litter layer was less extensive and the moss layer more prominent in sites with vital *P. braunii* populations as compared to sites where the species was decreasing or absent supports the conclusion that a continuous layer of tree litter reduces the safe sites required by *P. braunii* for establishment, and a closed litter cover in autumn and winter also suppresses the moss layer. A likely explanation of the different litter layer situation between sites with positive and negative population trends is that all sites with increasing populations are located in intact Tilio-Acerion forests, in which *Fraxinus, Acer* and other broad-leaved tree species with nutrient-rich and rapidly decomposing litter are prevailing, while stands with declining *P. braunii* populations are more often are found in forests with a significant contribution of *Fagus* which typically produces less nutrient-rich litter and thicker organic layers and may thus be less favorable for the species (cf. Jacob et al. 2010).

Does snow cover reduction in the course of climate change additionally threaten P. braunii?

A 50-yr record of snow cover has indicated that increasing winter temperatures and the reduced duration of snow cover in the course of climate warming may have played a role in the population decline. We found an annual average with days with snow cover of 110-170 in sites with increasing fern populations, 70-115 days in sites with decreasing populations and only 20-50 days in a region with only extinct populations (see Fig. 3.3. in chapter 3). Since sporophytes of *P. braunii* have been proven to be relatively frost hardy tolerating <-20 °C (Sato 1982), it is unlikely that the ferns are damaged by increased frost exposure in a climate with shorter snow coverage. Observations in potted plants, however, showed that leaves of *P. braunii* suffer from turgor loss during periods of soil frost and regain turgor in warmer winter periods. Consequently, frequent alternating frost and thaw events can cause excessive water loss. A closed snow cover would protect the species against suffering from frost desiccation in mild winter months, when their overwintering green fronds are exposed to an elevated atmospheric demand for water vapor while the thin soil is dried-out, still frozen or very cold. The fact that the sites of extinct populations had the shortest snow cover (84 d) of all sites investigated and that the location at which the ferns already disappeared 60 years ago (Saxon Uplands) is characterized by a mean snow period of only 50 d support the assumption that snow cover is important for *P. braunii* (cf. Eberle 1966, Mütter et al. 1998). Once again, the duration of snow cover has decreased during the last 50 years in all the regions with the species in Germany and may have deteriorated the growing conditions at the sites with already shorter snow cover duration in contrast to the situation several decades ago.

The effects of climate change on other rare and endangered ferns in Germany?

A review of the potential threats to the rare and endangered ferns in Germany (including members of the Polypodiopsida and Ophioglossaceae) based on information from the red list of the Federal Agency for Nature Conservation (Germany), Bennert (1999) and Jessen (2009) shows that for almost 50% of this group climate related reasons (in most cases periodic water scarcity) play a role for population declines. Similarities of certain habitat characteristics between single species might provide an explanation for this relationship. The rare and endangered ferns in Germany can be more or less separated into two different categories. About 20% occur in different kinds of wetland where interventions in the hydrology (e.g. drainage, river regulation, lowering of the ground water level) are thoroughly mentioned as principal causes of a threat. Other factors causing population declines are eutrophication or forest management (for ca. 60%) while climate aspects seem to play no important role for population losses within this group. The situation is very different regarding the other category which represents the majority of the endangered ferns in Germany (ca. 70%). The later almost consistently grow on shallow soils with little fine fraction or on sandy and coarse undergrounds where water storage capacity is limited, similar to the habitats of *P. braunii*. It is therefore hardly surprising that periodic water scarcity and drought damage during the summer month is mentioned as a serious problem for ca. 55% respectively 45% of this ferns (examples are *Asplenium adulterinum*, *Cryptogramma crispa*, *Asplenium cuneifolium*, *Asplenium fissum*, *Asplenium fontanum*, *Aplenium foresziensi*, *Asplenium obovatum ssp. lanceolatum*, *Woodsia alpina* and *Woodsia alvensis*). Once again, similar to *P. braunii*, spring droughts seem to be particularly problematic. In the case of *Ophioglossum vulgatum*, *Botrychium simplex*, *Botrychium lunaria* and *Botrychium matricariifolium*, for example, water scarcity among the sprouting period was found to cause a die off of single individuals before spore dispersal. While some species of habitats with shallow soils and small water holding capacity of the soil are highly desiccation tolerant (e.g. the sporophyte of *Asplenium ceterach*) it is possible that others will suffer even more from precipitation declines to come in future climates as it is suggested for *P. braunii* by the results of this study. Moreover, it can be speculated that with some species precipitation has fallen short of a certain threshold in the past as seems to be the case with *P. braunii* in the Saxon Uplands, with only extinct populations, and summer precipitation <400 mm.

Conclusions

Obviously, the striking association of positive population trends of *P. braunii* with higher air humidity and higher bryophyte cover may be coincidental, possibly masking the effect of other negative influences behind. However, the climate chamber experiment provides evidence that at least air humidity is indeed a critical factor for the survival of the species. The climate of Central Europe to come with precipitation and snow cover reduction and hotter and dryer summers (Beniston 2004, Schär et al. 2004, IPCC 2013) obviously contradicts the environmental needs of *P. braunii*. Moreover, other rare and endangered ferns seem to be affected, particularly those of habitats with shallow soils with only little water storage capacity. If litter type was indeed an important site factor for *P. braunii*, future changes in tree species composition with forest management could increase the vulnerability of the remaining populations. Since nowadays, all vital populations are found either in ravine forests with tree species producing rapidly decomposing leaves or in montane beech forests with the presence of maple, a conservation priority must be to preserve the current tree species composition and to halt any shift to beech-dominated stands in the Aceri-Fagetum stands.

The list of climatic, edaphic and stand structural parameters tested in our study is not exhaustive, and it is possible that other threating factor have been overlooked. Further investigation is required to gain a more comprehensive understanding of the ecology of woodland pteridophytes, both observational studies on the habitat requirements of the species and experiments in the laboratory and the field. One important question with respect to conservation measures concerns the shade tolerance of *P. braunii*. Our data indicate that the maintenance of a closed tree canopy might be an important prerequisite for the survival of the populations under drier summers in the future, because air humidity is typically reduced in canopy gaps. We further recommend carefully conducted small-scale experiments with manipulations of the moss and litter layers in the field for a better understanding of the role of forest floor cover with respect to the establishment and survival of *P. braunii* gametophytes and young sporophytes. A further urgent question would be how the sporophytes and gametophytes of *P. braunii* and other species respond to the reduction of snow coverage. It has also been assumed that a reduced snow cover threatens the endangered fern *Cryptogramma crispa* (Bennert 1999).

According to the main objectives of this study the following key findings can be summarized:

i) *P. braunii* appears to be an air humidity hygrophyte which, within the range of realistic environmental conditions set in the climate chamber experiment, suffers more from a reduction in RH than from a reduction in soil moisture

ii) Two factors may play a key role as drivers of population decline and local extinction of the German *P. braunii* populations: decreasing air humidity levels and a shift from a moss-covered to a litter-covered forest floor.

iii) Foliar water uptake may be widespread among temperate woodland ferns across different families, but it is largely dependent on the epidermal surface structure and functionality of the frond section.

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CHAPTER 6

Summary

Summary

This dissertation was enabled with a PhD grant to Robin Schwerbrock by Deutsche Bundesstiftung Umwelt (DBU). The study with different experiments in the laboratory and in the field aimed to exploring possible causes of the declines of *P. braunii* populations in Germany. Another objective was to develop a more comprehensive understanding of the ecology of temperate woodland ferns. A three factorial climate chamber experiment (temperature x relative air humidity x soil moisture) with 144 sporophytes of the species and realistic environmental conditions represents the first experimental proof for a soil moisture independent air humidity effect on the growth and the morphogenesis of a terrestrial woodland fern. By an observational study, we found hints that two factors may play a key role as drivers of population decline and local extinction of the German *P. braunii* populations; reduced air humidity levels and a shift from a moss-covered to a tree litter-covered forest floor as a consequence of climate warming or forest management are likely causes of decline in the German *P. braunii* populations. A tracer experiment with deuterated water further showed that foliar water uptake may be widespread among temperate woodland ferns across different families, but it is largely dependent on the epidermal surface structure and functionality of the frond section. The capability of absorbing liquid water via the epidermis has proven to be highest for *P. braunii* which is possibly caused by either active or passive effects of the species characteristic filamentous trichomes on the second-order pinnae. With respect to conservation of *P. braunii* in Germany, it must be a priority to preserve the current tree species composition of the remaining intact Tilio-Acerion stands with occurrence of the species and to halt any shift to beech-dominated stands. Further investigation will still be required which also concerns the ecology of the gametophyte generation of *P. braunii.* Yet, the results of this study may require correcting our conventional perception of the environmental controls of fern water relations focused on soil moisture.

CHAPTER 7

Appendix

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Declaration of originality and certificate of ownership

I, Robin Schwerbrock, hereby declare that I am the sole author of this dissertation entitled ‘Vulnerability analysis of the rare woodland fern *Polystichum braunii* (Dryopteridaceae) in Germany’. All data and references of this dissertation have been appropriately acknowledged. I declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

Göttingen, August, 08, 2016

(Robin Schwerbrock)